

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
11 July 2002 (11.07.2002)

PCT

(10) International Publication Number
WO 02/054052 A1

(51) International Patent Classification⁷: **G01N 27/12** MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(21) International Application Number: **PCT/US02/00461**

(22) International Filing Date: 8 January 2002 (08.01.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/260,250 8 January 2001 (08.01.2001) US

(71) Applicant and
(72) Inventor: FISH, Leonard [US/US]; P.O. Box 7891, Newport Beach, CA 92658 (US).

(74) Agent: CONNORS, John, J.; Connors & Associates, 1600 Dove Street #220, Newport Beach, CA 92660 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

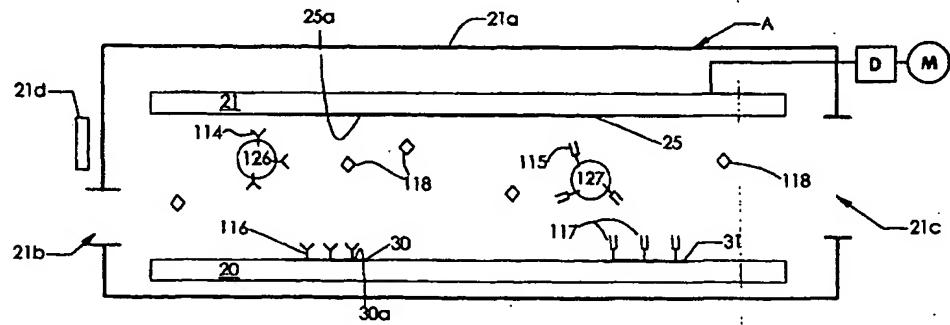
Published:
— with international search report

(48) Date of publication of this corrected version:
3 October 2002

(15) Information about Correction:
see PCT Gazette No. 40/2002 of 3 October 2002, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: DIAGNOSTIC INSTRUMENTS AND METHODS FOR DETECTING ANALYTES

WO 02/054052 A1

(57) Abstract: An instrument (A) detects in a sample the presence of an analyte. The sample includes electrically readable particles (126) with an agent attached thereto that binds with the analyte or is an analog of the analyte. The instrument includes a port for the sample and a pair of electrodes (25, 30), one of which has a surface portion (30a) with an agent thereat that binds with the analyte or is an analog of the analyte. The electrodes have a first position where they are separated a sufficient distance apart to enable the sample to move between the electrodes and a second position where the electrodes are in close proximity. A detection circuit, including the electrodes, has a first state when the analyte is absent and a second state when the analyte is present. A signaling device provides an indication of the state of the detection circuit.

**DIAGNOSTIC INSTRUMENTS AND METHODS
FOR DETECTING ANALYTES**

RELATED PATENT APPLICATION

This application is a PCT application based on U. S. provisional patent application Serial No. 60/260,250, entitled "Diagnostic Instruments And Methods For Detecting Analytes," filed January 8, 2001. This related application is incorporated herein by reference and made a part of this application.

DEFINITIONS

As used herein:

"analyte" means any molecule that is in a sample and is being assayed (analyzed);

"analog of an analyte" means a chemically modified analyte, such as, for example, an analyte molecule connected to a linker molecule and will not bind with the analyte;

"binding agent" means any molecule or group of molecules that are able to interact with an analyte;

"class of analyte" means a group of analytes that are detected by closely similar methods: There are five main classes of clinically relevant analytes: Class I proteins, Class II nucleotides such as, for example, RNA and DNA, Class III small molecules, Class IV electrolytes, and Class V cell detection;

"electrically readable particle or particles" means a particle or particles whose physical state, or presence or absence, can be determined through the use of an electronic circuit.

"electrolyte or electrolytes" means a compound or compounds that when dissolved in an aqueous medium dissociate into ions that make the medium conductive.

"small molecule or molecules" means a molecule or molecules that do not have binding sites and have, or may be modified to have, associated therewith an electrically detectable characteristic.

BACKGROUND OF THE INVENTION

A myriad of different clinical analysis methods for detecting the presence or absence of various classes of molecules (analytes) have been developed. These analysis methods are used widely in the biological field in detecting the presence of such molecules as proteins and other biomolecules. Proteins have been mainly analyzed by immunoassays such as RIA or ELISA format, DNA by gel or capillary electrophoresis after PCR amplification, small molecules such as glucose and cholesterol, by various color reactions, either chemical or enzymatic, and electrolytes such as sodium or chloride by ion sensitive electrodes. Recently, biochip or biodisc arrays have been developed for protein and DNA analysis. Instrumentation is widely different depending on the application.

In clinical laboratories and large hospitals hundreds of samples are processed with expensive and large automated analyzers. Smaller analyzers, such as microtiter well plate readers, are used in medium or small laboratories. The fastest growing market is the point-of-care (POC) market. Glucose and HCG (pregnancy test) are examples of well established tests in which, typically, strips or dipsticks are used.

Although a glucose test by necessity is quantitative, strip tests are qualitative or semi-quantitative at best with very limited dynamic range. The current technology does not allow quantitative assays *in vivo*, except for oxygen and possibly for glucose.

In all tests in which analyzers are used, the sample is taken first in a separate container such as a syringe or test tube or placed on a strip. Before actual assay, an aliquot of a sample or the strip is transferred into an analyzer. Transfer adds an inconvenient and potentially harmful step, because laboratory personnel can be exposed to pathogens. Although this problem has partially been solved by a cassette and applicator instrument designed for an optical disc based assay, even then a cassette must be inserted into an optical disc.

It would be highly preferable if a sampling device, the syringe or cassette, would be able to perform the actual assay immediately without any further transfer of a sample. Furthermore, it would be desirable to perform tens, or even hundreds, of different assays for various classes of analytes from the same sample. The instrument may be disposable, and of low cost.

SUMMARY OF THE INVENTION

This invention has several features. Without limiting the scope of this invention as expressed by the claims that follow, its more prominent features will now be discussed briefly. After considering this discussion, and particularly after reading the section entitled, "DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS," one will understand how the features of this invention provide its benefits, which include, but are not limited to, quick and accurate detection of analytes, either quantitatively or qualitatively, or both, elimination of exposure of health care workers to infection, rapid analysis of samples

containing multiple analytes, and provision of low cost disposable instruments or modules. In addition to application in the medical field, this invention may be used in connection with food and water safety measurements, military, and even anti-terrorist purposes.

The first feature is that the present invention solves most, if not all, of the problems associated with the prior art by a novel electronic/mechanical approach. It provides instruments and methods to detect the presence of a selected analyte in a sample including electrically readable particles having an agent attached thereto that binds with the selected analyte (Case I). In some embodiments of this invention, however, electrically readable particles need not be employed. These embodiments take advantage of a change in electrical characteristics between a pair of movable electrodes due to changes in their displacement with respect to each other (Case II). The electrodes may be formed by a photo-resist etching process or a plating process.

When electrically readable particles are used, they have binding agents at their surfaces. These binding agents bind with the analyte. They can be antibodies, oligonucleotides, or any other type of recognition molecule. The binding interaction between the analyte and the agent precedes detection of an electrical property, or a change in the electrical. Either the absence or the attendance of this electrical property, or a change in the electrical property, is sensed by a detection circuit which determines the presence or absence of the analyte. The electrical property may be current, resistance, conductance, inductance, capacitance, voltage, magnetic flux, or a phase shift. In accordance with the present invention, an electrically readable particle is bound to an electrode by an affinity binding in order to induce a significant change in the electrical properties of the detection circuit. The effect can be much greater than is obtained by binding an electro-active moiety onto a similar electrode, even if this

moiety is catalytic. Moreover, the binding of an electrically readable particle is universal and independent of the chemical nature of the binding pair.

Small molecules and electrolytes can advantageously be detected electrochemically by the present invention. Electrically readable particles are not used in assaying for small molecules and electrolytes. The embodiment of this invention that does this type of assay for either a specific small molecule or a specific electrolyte detects the absence or presence of an ion current using a pair of electrodes. One electrode has a surface portion that collects thereat the specific small molecule or electrolyte, as the case may be. This one electrode is at a first polarity. The other electrode has a surface portion that collects thereat small molecules or electrolytes other than said specific small molecule or electrolyte in question. This other electrode is at a second polarity opposite said first polarity. The electrodes have (i) a first position where they are separated a sufficient distance apart to enable the sample to move there between and to suppress the ion current and (ii) a second position where the electrodes are sufficiently close to each other to establish an ion current. In the first position the electrodes are separated a first distance apart sufficient to enable the sample to move between said electrodes and a second position where the electrodes are in close proximity to each other. The first distance is greater than the second distance.

There is a detection circuit, including the electrodes, that has a first state when the specific small molecule or electrolyte is absent from the sample and a second state when the specific small molecule electrolyte is present in the sample. A signaling device provides an indication when the detection circuit is in the second state.

Almost all clinical or chemical assays can be made using both Case I and Case II embodiments of this invention. Essentially all Class I

through V molecules identified above may be assayed. The molecules include, but are not limited to, peptides, proteins, glycoproteins, oligonucleotides, DNA, RNA, steroids, lipids, lipoproteins, carbohydrates, cathecolamines, several drugs, oxygen, nitric oxide, nitrous oxide, carbon dioxide, nitro, nitroso, azo, heterocyclic compounds, isocyanates, phenols, amines, most sulfur containing compounds, such as disulphides, dithiocarbamates, thiobarbiturates, thioureas, thiols, sulphonates, sulphides, and sulphoxides, and also cations, anions, chelates, and organometallics.

In the Case I embodiments of this invention, the detection circuit measures the electrical property produced when an affinity binding occurs between the analyte and the binding agent, for example, antigen-antibody interaction, DNA hybridization, ligand-receptor binding, enzyme-substrate, or enzyme-inhibitor interaction. The detection circuit includes an electrode, and preferably, uses at least one pair of relatively large surface area electrodes that in one position are widely separated, although sub-micrometer electrodes are still within the scope of this invention. Nevertheless, an individual sub-micrometer sized particle, or group of such particles, is large enough to fill the gap between the electrodes when these electrodes are in a second position in close proximity with each other to produce a detectable change in an electrical characteristic of the detection circuit.

One Case I embodiment of the present invention has a flow through capillary with a movable wall. The capillary contains two or more electrodes, at least one of which is connected to the movable wall. At least one electrode is coated with an agent that binds to the analyte. In the presence of the analyte, electrically readable particles having a binding thereon, such as a gold spheres, are bound onto the surface of the electrode coated with a binding agent. When two

electrodes are allowed to approach, so that both of them are in close proximity of the electrically readable particles, for example, a current is observed that is relative to the number of the electrically readable particles and the applied electric potential. Electrically readable particles will make the capacitor leaky. Thus, electrical properties can be changed by moving the electrodes in a controlled way into close proximity and measuring the electrical property change such as conductance, resistance, inductance, capacitance, or phase shift, in order to find out the modulation of these properties by an analyte. The instrument has a mechanism for bringing two electrodes into close proximity. The close proximity is obtained by providing an accurate moving mechanism and/or physical structures that prevent a direct contact between the electrodes and accurately space the electrodes apart a predetermined distance. This allows accurate electrochemistry to be performed quickly and at low cost.

Another Case I embodiment of the present invention comprises two opposing electrode arrays intersecting at right angles. Because the electric potential can be coupled between any pair of electrodes between two arrays, the number of high field areas, i.e., active working areas on electrodes can be much larger than the number of electrodes. A simple example is an orthogonal arrangement of two linear electrode arrays. Because two or more electrode arrays can be used, the number of tests can be quite large. The instrument in which multiple analytes are assayed simultaneously employs a microprocessor to record the electrical properties of each assay site independently, or a combination of assay sites that do not interfere with each other. This instrument is greatly simplified as compared with prior art devices employing electrode arrays and it facilitates the construction of massive, very low cost arrays.

The present invention enables, perhaps for the very first time, a disposable self-contained instrument that is able to perform hundreds, or even thousands of tests qualitatively, quantitatively, or both, and quickly from a very small amount of sample. It solves most problems associated with the prior art. The assay can be performed in the sample collection instrument. No transfer or aliquoting of the sample is required. Results are obtained fast and they are quantitative. The detection is very sensitive, because a single electrically readable particle can be detected, and only one analyte molecule is necessary for the binding of that particle. The electrode arrays may allow multiple tests to be performed from each sample. The instrument may incorporate a low cost microprocessor and display, or be inserted into an adapter for connection to a computer having a microprocessor and display.

All these advantages combined enable construction of a self-contained disposable instrument that is able to analyze quantitatively, sensitively, and quickly several different analytes simultaneously. The instrument can also be modular, so that only the sample collection and measuring unit is disposable, while processing and display units are used repeatedly, or it can be a single use, disposable hand held instrument used for specific tests, or a computer equipped with an adapter.

In a third embodiment of the instrument of this invention, disposable modules, or self-contained assay instruments for the Point-of-Care, are provided. The modules can have sample collection and processing equipment including, for example, cell separation, cell lysis, reagent storage and mixing, and analyte fractionation, such as chromatography and electrophoresis. The modules have the electrodes for actual measurement. These modules can be used in combination with a processing and display instrument that has been designed for

this purpose or modules can be connected via an adapter to a computer, such as a personal computer (PC). Self-contained disposable instruments may also have processing and display capability. The information can be transferred to a permanent storage unit, such as PC hard drive, before the disposal. The measurement modules, which have a sample inlet port, are in a rack or in a conveyer belt. A pipetting robot will add samples to each module, and after a short incubation time another robot will put each module into a data collection and processing system.

This invention includes several methods. Broadly, the method of this invention detects in a sample the presence of an analyte. It includes the steps of

(a) introducing into the sample electrically readable particles with an agent attached thereto that binds with the analyte or is an analog of the analyte,

(b) providing an instrument including

a pair of electrodes, said electrodes having a first position where they are separated a first distance apart sufficient to enable the sample to move between said electrodes and a second position where the electrodes are in close proximity to each other, said first distance being greater than said second distance,

a detection circuit, including the electrodes, that has a first state when the analyte is absent from the sample and a second state when the analyte is present in the sample, said second position determining the state of the detection circuit,

a signaling device that provides an indication of the state of the detection circuit with the electrodes in the second position,

(c) contacting the sample, including the electrically readable particles, with the electrodes while said electrodes are in the first position,

(d) removing any unbound particles from between the electrodes, and

(e) moving the electrodes to the second position.

In another method the electrically readable particles are stored on a holding electrode. These stored particles may or may not carry a binding agent. If they do not, they arrived independently at the surface of this electrode through electrostatic attraction. By reversing either the charge of the particles, or of the electrode, the non-specifically bound particles are removed, while specifically bound particles remain bound.

DESCRIPTION OF THE DRAWING

The preferred embodiments of this invention, illustrating all its features, will now be discussed in detail. These embodiments depict the novel and non-obvious instrument and methods of this invention as shown in the accompanying drawing, which is for illustrative purposes only. This drawing includes the following figures (Figs.), with like numerals indicating like parts:

Figs. 1A and 1B are schematic illustrations of a first embodiment of the instrument of this invention depicting test sites where analytes in a sample are individual molecules with at least two spaced binding sites.

Figs. 2A and 2B are schematic illustrations of a second embodiment of the instrument of this invention, which is similar to the first, depicting test sites where DNA specimens are the analytes in the sample.

Figs. 2C through 2G are schematic illustrations of the second embodiment of the instrument of this invention depicting using nano tubes as the conductive particles, wherein

Fig. 2C shows a pair of spaced apart electrodes with nano-tubes in a sample that is between the spaced apart electrodes,

Fig. 2D shows the electrodes of Fig. 2C moved into close proximity with each other and the analyte attached to the left handed assay site,

Fig. 2E is a side elevational view of a nano-tube.

Fig. 2F is an illustration of an end of the nano-tube being chemically treated to form thereat a binding site,

Fig. 2G is a cross-sectional view taken along line 2G-2G of Fig. 2E.

Figs. 3A and 3B are schematic illustrations of a third embodiment of the instrument of this invention including an electrode that has at least one electrical property in a detection circuit that (a) does not change because the analyte attaches to the agent on the electrically readable particles and (b) that changes in the absence of the analyte due to the agent on the electrically readable particle binding to the analog of the analyte on the electrode.

Figs. 3C and 3D are schematic illustrations of another version of the third embodiment of the instrument of this invention where an analog of the analyte is bound to the particle.

Fig. 4 is a schematic illustration of the fourth embodiment of the invention showing an assay site for detecting electrolytes.

Fig. 5 is a schematic illustration of another version of the fourth embodiment of the invention showing an assay site for detecting small molecules such as glucose.

Fig. 6A is a schematic illustration of a fifth embodiment of the instrument of this invention shown in a plan view.

Fig. 6B is a cross-sectional view taken along line 6B-6B of Fig. 6A showing a capillary structure in an open position.

Fig. 6C is an enlarged fragmentary view taken along line 6C of Fig. 6B.

Fig. 7A is a cross-sectional view similar to that shown in Fig. 6B but with the capillary structure in a closed position.

Fig. 7B is an enlarged fragmentary view taken along line 7A of Fig. 7.

Fig. 8A shows cross-sections of different capillary structures.

Fig. 8B is a cross-sectional view of a capillary structure with two separate walls, one being moveable relative to the other.

Fig. 9 is a plan view of a sixth embodiment of this invention employing a grid of overlying orthogonally oriented electrodes creating at the overlying intersections of the electrodes an array of test sites.

Fig. 10 is a perspective view of one of the test sites created by the overlying orthogonally oriented electrodes shown in Fig. 9.

Figs. 11A through 11G are schematic illustrations depicting the steps employed in making the grid shown in Fig. 9.

Fig. 12 is a plan view of an array of assay sites having a holding electrode in advance of the array.

Fig. 13A is a cross-sectional view of a seventh embodiment of this invention schematically illustrating an instrument employing electrodes coated with a thin layer of soft material and the electrodes in an open position.

Fig. 13B is a cross-sectional view of the seventh embodiment of this invention schematically illustrating the instrument shown in Fig. 13A with the electrodes in a closed position.

Fig. 14A is cross-sectional view of an eighth embodiment of this invention schematically illustrating the use of a flexible, resilient electrode.

Fig. 14B is a plan view taken along line 14B-14B of Fig. 14A.

Figs. 15A through 15C are cross-sectional views of a ninth embodiment of this invention schematically illustrating an instrument employing a roller moving past different assay sites.

Figs. 16A through 16D are cross-sectional views of a tenth embodiment of this invention schematically illustrating a hand held, single use, portable, disposable instrument with a needle that is extended to collect a sample of blood from a subject and retracted after collecting the sample.

Fig. 16E is a plan view of the instrument shown in Figs. 16A through 16D.

Fig. 16F is a plan view of a display on the instrument shown in Figs. 16A through 16D providing a qualitative readout that a sample is positive for E. Coli in the sample and a quantitative readout indicating the amount present in the sample.

Fig. 17 is a schematic illustration of a gold particle coated with a resistive material.

Figs. 18A through 18D show an eleventh embodiment of this invention employing a disposable module used with the portable, reusable, computer adapter shown in Figs. 19A and 19B and is used with the computer shown in Fig. 20, wherein

Fig. 18A is a cross-sectional taken along line 18A-18A of Fig. 18B,

Fig. 18B is a cross-sectional taken along line 18B-18B of Fig. 18A,

Fig. 18C is a cross-sectional taken along line 18C-18C of Fig. 18B,

Fig. 18D is a cross-sectional taken along line 18D-18D of Fig. 18B.

Fig. 19A is a cross-section view of a computer adapter to be inserted into a USB port of the computer shown in Fig. 20, showing the disposable module of Figs. 18A through 18D about to be inserted into the computer adapter.

Fig. 19B is a cross-section view similar to that of Fig. 19A showing the disposable module of Figs. 18A through 18D inserted into the computer adapter.

Fig. 20 is a schematic illustration of a computer network with the assembly of the disposable module and computer adapter shown in Fig. 19B being connected to the USB port in a computer.

Figs. 21A through 21C depict schematically the twelfth embodiment of this invention showing a sample carrier to be used with the automatic testing instrument shown in Fig. 21 wherein

Fig. 21A is a plan view of the sample carrier comprising the top plate shown in Fig. 21B and the bottom plate shown in Fig. 21C,

Fig. 21B is a plan view of the top plate of the sample carrier, and

Fig. 21C is a plan view of the bottom plate of the sample carrier.

Fig. 22 is a plan view of an automatic detection system for testing numerous samples on a sustained basis over a prolonged period of time.

Fig. 23 is a detection circuit employed in this invention where changes in inductance, resistance and/or capacitance are detected.

Fig. 24 is a detection circuit employed in this invention where changes in voltage are detected.

Fig. 25 is a detection circuit employed in this invention including a multiplexer.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

First Embodiment

Fig. 1 depicts an instrument A including a pair of conductive electrodes 25 and 30, respectively mounted on insulator base support members 21 and 20. The electrodes 25 and 30 have substantially flat, planar surfaces 25a and 30a facing each other and each lying in

different parallel planes. These electrodes 25 and 30 are directly opposed to each other vertically, and each have an area that exceeds about 0.5 square micrometer, and typically, each have an area of from about 10 square micrometers to about 10 square millimeters. The electrodes 25 and 30 are shown in a spaced apart position in Fig. 1A and, after introducing a sample and then removing unbound material from between the electrodes 25 and 30, moved to the dotted line position shown in Fig. 1B. As shown in Fig. 1B schematically, the electrodes 25 and 30 are brought into close proximity, almost but not quite touching. Typically, the distance between the electrodes 25 and 30, when in the position shown in Fig. 1A, is from about 5 micrometers to about 5 millimeters and, when in the position shown in Fig. 1B, is from about 10 nanometers to about 5 micrometers. A third electrode 31, spaced from the electrode 30, and also mounted on the base support 20, provides a second assay site. Binding agents 116 and 117, are respectively on the surfaces of the electrodes 30 and 31. As discussed further in connection with the embodiments shown in Figs. 9 through 12, a plurality of pairs of directly opposed electrodes form numerous assay sites that enable an instrument according to this invention to detect several different analytes in any sample.

The base support members 21 and 20 are enclosed within a housing 21a having an inlet port 21b providing an opening in the housing through which a sample is introduced into the instrument A and an outlet port 21c through which sample flows from the housing. The port 21a may have a cover 21d that is moveable between open and closed positions. As illustrated, the cover 21d is shown in an open position. The sample includes electrically readable particles 126 and 127 and binding agents, the antibodies 114 and 115, are bound respectively to the surfaces of these electrically readable particles 126 and 127. The electrically readable particles are introduced into the

sample prior to, during, or after the sample is fed into the instrument A. In this illustration, the sample also includes an analyte 118, for example, insulin. This analyte 118, having at least two spaced apart binding sites, only reacts with the binding agent 116 on the surface of the electrode 30. Upon introduction of the sample, including the particles 126 and 127 and the analyte 118, into the instrument A between the electrodes 25 and 30, the analyte attaches to the particles 126 and to the binding agent 116 on the surface of the electrode 30. Thus, on the left side, the first assay site, the electrically readable particle 126 on the electrode surface forms an antigen conductor. No affinity binding occurs on the right side, the second assay site, with the particle 127, because there are no analytes present that bind with both the binding agents 115 and 117.

In accordance with this invention, the directly opposed pairs of electrodes, electrodes 25 and 30 and electrodes 25 and 31, are moved from the position shown in Fig. 1A to the position shown in dotted line in Fig. 1B. A motor M having its output shaft (not shown) is connected to a drive mechanism D that, upon energizing the motor, moves the support member 21 downward towards the other electrodes 30 and 31 on the support 20. In the position shown in Fig. 1A, the pair of electrodes 25 and 30 is separated a sufficient distance to allow the sample to flow between this pair of electrodes and out the outlet port 21c. After an adequate time period has elapsed to allow binding to occur if the analyte 118 is present, all the assay sites are washed, for example with distilled water, to remove unbound material. An alternately mechanism such as depicted in Fig. 12 may be used. This mechanism shown in Fig. 12 will be discussed subsequently in greater detail. After washing, the motor M is energized to move the electrode to the dotted line position shown in Fig. 1B. Preferably, pressure is applied to the particle 126 bound to the electrode 30. This is

especially important when the detection circuit responds to changes in conductivity. The pressure is controlled. It must not be so great as to cause a short circuit between the electrodes 25 and 30, yet sufficient to break surface tension. The application of pressure is carefully controlled so that the electrodes pairs of an assay site do not touch, but will insure electrical contact between the particles 126 bound to the electrode 30 and also between these bound particles and the electrode 25. As discussed subsequently, a spacer device may be employed to insure that the electrodes do not touch and short out the detection circuit. When the detection circuit responds to changes in inductance, capacitance, or phase shift, the application of pressure is not critical.

The pairs of electrodes 25 and 30 are components in an electrical circuit such as, for example, the detection circuits depicted in Figs. 23 through 25. These detection circuits each have a first state when the analyte is absent from the sample and a second state when the analyte is present in the sample, and a signaling device, for example, a liquid crystal display (LCD) that provides an indication when the detection circuit is in the second state. The signaling device provides a qualitative read-out identifying the analyte, or a quantitative read-out corresponding to the amount of analyte in the sample, or both. These detection circuits will be discussed in greater detail subsequently.

Because very small electrically readable particles 126 and 127 are used, the instrument A is very sensitive. It can even detect a single molecule of the analyte 118. For example, a cancer cell will produce a protein molecule or molecules that are indicative of the presence of cancer cells. In the early stages of the cancer, only very low levels of this marker protein molecule are in a sample of a subject's blood. Conventional testing techniques are not usually capable of detecting such low concentrations of these identifying protein molecules. The

attachment of a single marker molecule to an electrically readable particle that is in the presence of an electrode at an assay site creates a change in state in the detection circuit that indicates the presence of this single molecule.

Second Embodiment

The second embodiment of this invention, the instrument B shown in Fig. 2, is similar to that shown in Fig. 1, but the housing 21a and motor M and drive mechanism D are not shown. This embodiment is a schematic representation of a DNA test. The electrodes 30 and 31 are coated with different probes, that is binding agents, respectively probes 216 and 217. The analyte or target 218 is able to bind with the probe 216 as well as with a probe 214 on the surface of the electrically readable particle 226. The probes 215 and 217 do not bind with the target 218. As discussed above in connection with Figs. 1A and 1B, after binding of the target 218, unbound material is removed from the space between the electrodes 25 and 30 and the electrodes are moved towards each other and a detection circuit is then activated.

Figs. 2C through 2G schematic represent another DNA test. In accordance with one aspect of this invention, as shown in Figs. 2C through 2G, the conductive particles may be nano-tubes 26, such, for example, manufactured by Carbon Nanotechnologies, Inc. of Huston, Texas. Nano-tubes 26 are graphite-like elongated tubes having either opened or closed ends and hallow interiors with an outside diameter D of from about 1 nanometer to about 50 nanometers and a length L of from about 10 nanometers to about 10 micrometers. As shown in Fig. 2G, the nano-tubes 26 have an inside diameter D1 of from about 10 to 20 nanometers, with a wall thickness of no less than a single atom.

As illustrated in Fig. 2F, the opposed ends 26a and 26b of the nano-tubes have been reacted with such chemical reagents as a mixture

of osmium tetraoxide and periodic acid, bromine, chromic acid, or potassium permanganate, to form reactive groups. When the mixture of osmium tetraoxide and periodic acid is used the reactive groups are oxygen sites. These oxygen sites are reacted with amino terminated oligonucleotides R to form at the opposed ends binding sites 26a and 26b. These binding sites on the nano-tubes 26, in the presence of the analytes 15a and 15b, which must both be present, bind with the analytes at the right hand assay site shown Figs. 1C and 1D. There are binding agents 16 and 16a, respectively at the surfaces of the electrodes 20 and 21 that bind with the analytes attached to one nano-tubes 26. The analytes 18 do not bind with the binding agents 17 and 17a at the electrodes 21 and 20 at the right hand assay site shown in Figs. 1C and 1D. To make the nano-tubes 26 soluble they are coated with a detergent such as tween-20. In accordance with one aspect of this invention, the electrodes 20 and 21 are more into closed proximity with each other as shown in Fig. 1D prior to measuring for attachment of the analytes to an assay site. A reference discussing chemical modification of the ends of the nano-yubes is discussed by S> S> Wong et al in Covalently-Functionalized Single-Walled Carbon Nanotube Probe Tips for Chemical Force Microscopy, J. Am. Chem. Soc. 1998, 120, 8557-8558.

Third Embodiment

In Figs. 3A and 3B, a competitive or inhibitory assay is illustrated using a third embodiment of this invention, the instrument C. The instrument C is similar to that shown in Fig. 1, but the housing 21a and motor M and drive mechanism D are not shown. In this third embodiment, analogs of analytes 316 and 317 are attached respectively to the surfaces of the electrodes 30 and 31, respectively. In the presence of the analyte 118 in the sample, binding to the electrically readable particle 126 is diminished. Failure of the particle 126 to bind

to the first assay site due to the presence of the analyte 118 is an indication that the analyte 118 is in the sample and the lack of binding will be detected by a detection circuit. In contrast, the particle 127 is bound to the analog 317 on the electrode 31 at the second assay site.

Figs. 3C and 3D depict the particle 126 with the analog of the analyte 316 on the surface of the particle and the binding agents 116 on the electrode 30. In this case, when the electrodes 25 and 30 are moved from the spaced apart position shown in Fig. 3C into close proximity as shown in Fig. 3D, the analyte 118 does not bind to the particle 126, but will bind to the binding agents 116 on the electrode 30. The detection circuit detects the absence on the electrode 30 of the particle 126 having thereon the attached analyte 118.

Fourth Embodiment

As shown in Figs. 4 and 5, electrolytes and small molecules, are assayed using Case II embodiments of this invention. Fig. 4 depicts an instrument C1 for assaying a specific electrolyte, and Fig. 5 depicts an instrument C2 for assaying a specific small molecule. In Fig. 4 the electrode 30 has on its surface a membrane 418c can only be penetrated due to osmosis by potassium ions (K+) 418b. Other ions present such as the chloride ion (Cl-) 418a can not penetrate this membrane 418c. This is known technology. In accordance with this invention, the electrodes 25 and 30 are initially spaced widely apart as shown in solid lines. After sufficient time has elapsed to establish an ion current created by the movement of the potassium ions (K+) 418b and chloride ions (Cl-) 418a, respectively to the electrodes 30 and 25 due to the electrodes' opposite polarities, the electrodes are moved together into the position shown in dotted lines to suppress this ion current.

As depicted in Fig. 5, as is well known, a small molecule such as, for example, glucose 518, in the presence of hydrogen peroxide (H_2O_2) 519, will bind employ enzymes 516 to hold them to the surface of the electrode 20. In a similar manner as discussed in connection with the instrument C1, there is an ion current is first generated in the instrument C2, which ion current is suppressed by changing the position of the electrodes 21 and 21 from that shown in solid line to that shown in dotted lines.

Fifth Embodiment

As illustrated in Figs. 6A-6C, 7A and 7B, and 8A and 8B an instrument D using a capillary tube 641 to move a pair of electrodes 625 and 630 between open and closed positions. This capillary tube 641 has upper and lower halves 620 and 621. The upper half 620 carries on its inside surface the one electrode 625. The lower half 620 carries on its inside surface the other electrode 630. As shown in Fig. 6B, the two halves 621 and 620, when separated as shown, provide a channel 601 through the capillary tube 641. Typically, the channel 601 has a diameter of from about 10 micrometers to about 10 millimeters. The tube 641 is preferably made of a plastic such as, for example, polystyrene, polypropylene, a polyimide, or a polyflorocarbon such as Teflon™. Both electrodes 625 and 630 are parallel with the longitudinal axis of the capillary tube 641 and are essentially identical to the electrodes depicted in Figs. 1A and 1B. The electrode 630 is coated with a binding agent as discussed previously in connection with Figs. 1A and 1B, and also sparsely coated with insulator (plastic) spherical spacer beads or particles 626. These spacer particles 626 are of smaller diameter than the electrically readable particles 126 in the sample flowing through the capillary tube 641. Typically, the electrically readable particles 126 have a diameter at least 10 percent

greater than the diameter of the spacer particles 626. For example, if the spacer particles 626 are 200 nanometers, the electrically readable particles 126 are 400 nanometers.

There are pins 618 and 619 respectively in the contact with the electrodes 625 and 630 for electrically connecting these electrodes in a detection circuit. The cross-sectional configuration of the channel 601 is oval. The capillary tube 641 may have cross-sectional configurations other than oval. As shown in Fig. 8A such other cross-sectional configurations are, for example, circular 1, eye-shaped 2, rectangular 3, rhombic 4, and crescent 5. As depicted in Fig. 8B, the capillary tube 641 may have two walls 6 and 7 that are moveable relative to each other.

The capillary tube 641 is made by bonding together the two similarly shaped halves 620 and 621, but the lower half 621 has a 250 nanometer (nm) deep and 200 μm wide longitudinal trench that is coated with a thin gold layer forming the electrode 630 in contact with the pin 618. The upper half 621 of the capillary tube 641 is also partially coated with a thin gold layer forming the electrode 625 in contact with the other pin 619. The electrode 621 is coated with binding agents 116 (Fig. 1A) and sparsely coated with 200 nm nonconductive spherical particles 626. The purpose of these particles 626 is to act as spacers that prevent the two electrodes 625 and 630 touching each other when the capillary tube 641 is compressed.

When sample flows through the channel 601 capillary tube 641, it carries with it 250 nm gold particles 126 that are coated with the secondary binding agent 114. The number of the gold particles that are attached to the electrode 630 depends on the concentration of the analyte. The pins 618 and 619, for example, are in a detection circuit including an electric potential source. Any unbound gold particles 126 are washed away either with the sample or by a wash buffer. The

capillary tube 641 is then compressed as shown in Figs. 7A and 7B, so that both electrodes 625 and 630 are in contact with the particles 126. Because of the diameter of the gold particles 126 is greater than the diameter of the nonconductive spherical particles 626, an electrical property, in this case a current flow, is only produced when the gold particles 126 are present. A DC current will be directly proportional to the number of the bound gold particles. Due to the small distance between the electrodes 625 and 630 during compression of the capillary tube 641 any ionic current will be only transient, provided the potential is not too large to induce electrochemical reactions.

Many capillary tubes 641 may be assembled into an array. The height of the uncompressed channel 601 is more than the diameter of the electrically readable particles 126, that is, between 1 nm and 1 millimeter (mm), and preferably 10 nm and 3 micrometers (μm). The minimum width of the channel 601 is more than the width of an electrode, preferably at least 2 times the width of an electrode. The maximum width is less than a hundred times the width of the electrode 630, which is coated with the binding agent. The width of one electrode 625 or 630 varies, for example, between 100 nm and 10 mm. An array of capillary tubes 641 may contain up to 1000 electrodes. The large arrays tend to have smaller electrodes than small arrays. Thus, the total width occupied by an electrode, or an electrode array, is typically between 100 nm and 20 cm. The length of a capillary tube 641 depends on the number and type of various assays, and the accuracy that is required. The length varies between 100 μm and 1 meter (m), and is preferably 5 mm-50 mm.

Although in its simplest embodiment the present invention has no capillaries, or just one capillary, much more sophisticated microfluidic structures are possible. Reagents can be stored in cavities that are closed by a metal or plastic film that will be dissolved

electrochemically just before the assay. The analytes can be fractionated by various chromatographic methods, including, affinity, size exclusion, ion exchange, adsorption, and reverse phase chromatographies. Electrophoresis is another well known technique for the separation of biomolecules. All these methods are well known in the art. Examples of microfluidic systems are: U. S. Patent No. 4,426,451 of Columbus, entitled Multi-Zoned Reaction Vessel Having pressure-Actuable Control Means Between Zones; U. S. Patent No. 4,753,776 of Hillman et al, entitled Blood Separation Instrument Comprising A Filter And A Capillary Flow Pathway Exiting The Filter; U. S. Patent No. 4,855,240 of Rosenstein et al, entitled Solid Phase Assay Employing Capillary Flow; U. S. Patent No. 4,963,498 of Hillman et al, entitled Capillary Flow Instrument; U. S. Patent No. 5,304,487 of Wilding, entitled Fluid Handling In Mesoscale Analytical Instruments; U. S. Patent No. 5,698,406 of Cathey et al, entitled Disposable Instrument In Diagnostic Assays; U. S. Patent No. 5,798,215 of Cathey et al, entitled Instrument For Use In Analyte Detection Assays; U. S. Patent No. 5,714,390 of Hallowitz et al, entitled Cartridge Test System For The Collection And Testing Of Blood In A Single Step.

An important part of the present invention is a movable electrode. In the preferred embodiment, at least two electrodes are inside the capillary tube 641, so that by applying a force the walls of the two halves 621 and 620 of the capillary tube come into close proximity. The capillary tube can be compressed to bring the electrodes 625 and 630 close to each other by many different means as discussed subsequently. In mass fabrication it is easier to evaporate or sputter the electrodes onto the inside surfaces of the halves 621 and 620 prior to bonding them together to form the capillary tube 641. One part is treated chemically to attach the binding molecules and then the two halves 621 and 620 are bonded together. However, by laminar

etching techniques it is possible to perform etching inside a capillary tube and also to attach binding molecules. Although the bonding of two halves 621 and 620 is eliminated, this approach is relatively slow and is not currently preferred in mass production. Capillary tubes 641 can also be assembled from more than two parts. For example, each electrode and associated binding molecule can be deposited on a separate substrate. These are cut into flexible strips placed inside a capillary tube or assembled into arrays.

There is no absolute need to bond the recognition electrode and the movable electrode into one fixed capillary tube. The movable electrode can fit into a slot, in which it can slide and come into contact with the electrically readable particles. This is depicted in Fig. 8B. The contact surfaces between the two parts are preferably hydrophobic to prevent the leakage of the sample. If the sample volume is very high and not considered dangerous, the electrodes can be on an open surface. For example, when drinking or swimming water is tested, an open and relatively large area recognition electrode array is put inside water. After certain incubation time it is taken from the water and electrically readable particles 126 are added in suspension. After incubation, any unbound particles 126 are washed away, and the movable electrode array is brought into a close proximity and the measurement is performed. However, in clinical applications, capillary tubes are currently preferred in order to keep pathogens contained within them.

Many types of materials may be used in making the capillary tube 641, with the body of the halves 620 and 621 serving as substrates. If the halves 620 and 621 are not bonded together, there is no need for them to be deformable. If they are bonded into one structure as depicted in Fig. 6B, at least one half must be flexible. The materials are

preferably non-conductors, although one half may be conducting. Accordingly, almost any material can be used, including but not limited to, metals, semiconductors, photoconductors, silicon oxide, ceramics, glass, rubber, plastics, such as polyethylene, polypropylene, polycarbonate, polyvinylchloride, polybutadiene, polystyrene, polyacetal, and silicone. This material may also be used in constructing the electrodes in any of the other embodiments disclosed. Substrates and patterning are discussed in the following references: United States Patent Nos. 4,357,311; 4,886,761; 4,959,303; 5,514,501; 4,302,530; 4,562,157; and 5,413,732.

In Fig. 6B the capillary tube 641 is open. When the sample is drawn into this tube 641, the tube is compressed virtually into a closed state during the measurement. The situation can be reversed in the sense that the resting state of a capillary tube can be closed and it is expanded to be open during introduction of the sample. This approach has some advantages. First, the instrument acts like a Vacutainer™ (Becton Dickinson) with the difference that in this case the vacuum is created just before the sample is drawn. Second, because both surfaces can be flat, the fabrication is easier. A pre-drawn vacuum could be utilized, but it might affect the shelf life, and other variables.

Sixth Embodiment

Figs. 9 and 10 illustrate the sixth embodiment of this invention, an instrument E including inlet and outlet ports that allow a sample to flow past an electrode array EE. In this embodiment five electrodes 30-34 are supported on the surface of a lower insulator substrate as discussed in connection with Figs. 11A through 11G and similar to that shown in Fig. 1A and three electrodes 40-42 are supported on the surface of an upper insulator substrate as discussed in connection with Figs. 11A through 11G and similar to that shown in Fig. 1A. There are

total fifteen intersections, which have coordinates (30,40), (30,41), (30,42), (31,40) (34,40), (34,41), and (34,42). A different assay can be performed in each intersection to detect different analytes. Binding agents specific to the analyte to be detected at an individual assay site are on the surfaces of the electrodes 30-34 at each site, and electrically readable particles 126 are in the sample being tested as discussed above. Preferably, spacer particles 626 are at each site as shown in Fig. 10.

Figs. 11A through 11G illustrate the manufacture of the electrode array shown in Fig. 9. Conventional manufacturing techniques are employed. As shown in Fig. 11B, an insulator substrate 20 with five pins 35-39 extending outward from an edge 20a of the substrate has a conductive layer 48 deposited of the substrate's surface so that electrical contact with pins 35-39 is established. As shown in Fig. 11C, the conductive layer 48 is covered with a photoresist 49. As shown in Fig. 11D, after illumination with ultraviolet light through a mask (not shown) extra photoresist 49 is removed, and electrode strips 50-54 are formed by etching away the exposed conductor 48. As shown in Fig. 11E, the remainder of the photoresist 49 is removed, and the electrodes 30-34 are exposed. As shown in Fig. 11F, the electrodes 30-34 are coated with another photoresist 55. As shown in Fig. 11G, the final pattern 59 is created illuminating through an appropriate mask and dissolving away the soluble part of the photoresist 55. This fabrication method gives an electrode array, in which the electrodes are exposed only in the intersections, thus creating the assay sites at each intersection. In a simplified approach only steps shown in Fig. 11A through Fig. 11E are used.

Fig. 12 shows the electrode array illustrated in Fig. 9 having a holding electrode 61 in advance of the array. By alternating the electrical voltage applied to this electrode 61 electrically readable

particles 126 being held to the surface of this electrode may be released and mixed with sample flowing through the instrument's inlet port (not shown). Before the electrodes at each assay site have been moved into close proximity with each other, the voltage applied to the holding electrode 61 is changed to remove unbound particles 126. The holding electrode 61 may be prepared exactly in the same way as discussed above in connection with Figs. 11A through 11G.

Seventh Embodiment

As depicted in Figs. 13A and 13B, a seventh embodiment of this invention, the instrument F, similar to the instrument A shown in Fig. 1A, employs the spaced apart electrodes 25 and 30. Each electrode has a thin layer 22 and 23, respectively, of soft material covering it. This soft material may be, for example, silicone plastic. The thickness of the soft layer should be about half diameter of the electrically readable particle 126, for example about 100 nanometers when using a electrically readable particle having a diameter of a little greater than 200 nanometers. The lower binding agent 116 is applied to the surface of the layer 23. Thus, in accordance with this invention, the binding agent 116 need not be applied directly to the surface of the lower electrode 30, but only be nearby this surface, or as expressed in the claims "thereat." When the electrodes 25 and 30 are moved from their widely spaced apart position as shown in Fig. 13A into close proximity with each other as shown in Fig. 13B, and pressure is applied to the electrodes, the particle 126 is pushed through the layers of 22 and 23 and binding occurs in the presence of the analyte 118, as discussed above. Thus, the layers 22 and 23 act as spacer elements.

Eighth Embodiment

As illustrated in Figs. 14A and 14B, a eighth embodiment of this invention, the instrument G, employs a roller 65 having its shaft 66 connected to a drive mechanism (not shown) that rolls the roller over three assay sites 61-63 sequentially, each site including pairs of electrodes. The upper electrode 64 is common to all three sites 61-63, and it is made of a flexible material, so that it bends as the roller 65 passes a site to move this electrode into close proximity to the lower electrodes 30 through 34 at any given site 61, 62, or 63. The roller 65, as shown in Fig. 14B, extends across all the lower electrodes 30 through 34, to which particles 126 are attached when analyte is present in a sample. A timing strip 34 is positioned along the path of travel of the roller 65, which engages markers 34a and 34b disposed in spaced apart positions along the timing strip to provide an electrical signal indicating the position of the roller relative the assay sites 61-63. Upon passing a site, the electrode 64, being resilient, returns to its widely spaced apart position.

Ninth Embodiment

The ninth embodiment of this invention, the instrument H, employs a ball electrode 73 between two electrodes 71 and 72, respectively on non-conductive substrates 21 and 20. Affinity bound electrically readable particles 126 are in wells 71a defined by a thin patterned spacer layer 59 of insulating material. The ball electrode 73, being conductive, will serve as an extension of the upper electrode, and in effect, constitutes moving a pair of electrodes between a first spaced apart position to a second position where the electrodes are in close proximity.

Tenth Embodiment

Figs. 16A through 16B depict the tenth embodiment of this invention, a self-contained single use analyzer instrument I that is disposable and is hand held. This instrument I includes a housing 82a having a U-shaped cross-section with an orifice 82 in a side wall. Embedded within the base of the housing 82a are a microprocessor device 82b and memory device 82c used to control the operation of the instrument I. Seated within the housing 82a is a moveable block 83a holding a needle 83 that is manually moveable between a retracted position shown in Fig. 16A and a sample collection position shown in Fig. 16C. Within the block 83a is an electrode array EE providing a plurality of assay sites such as illustrated in Fig. 12, a battery 84, a filter 87 on the inlet side of the electrode array EE, and a pad 88 of absorbing material such as cotton. An external handle 80 attached to the block 83a is used to move manually the block from within the housing 82a as shown in Figs. 16A and 16B to an outwardly extended position shown in Fig. 16C, and then return the block to the position shown in Fig. 16D. There is a lever 81 extending through the block 83a that is used to return manually the needle 83 to the position shown in Fig. 16D. This lever 81 has an internal end connected to the basal end of the needle 83 and an opposed end extending from the block 83a. A powerful spring 86 is coiled about the basal end of the needle 83 that force the needle through the orifice 82 when the needle is aligned with this orifice as shown in Fig. 16B and 16C. A pellet 6 of sugar, or other water soluble bio-compatible material, containing the electrically readable particle 126 is positioned on the base of the housing 82a upstream of the array EE.

In Fig. 16A the instrument I is in a ready to use condition and the needle 83 is enclosed entirely within the housing 82a. First, the instrument I is pressed against the skin of the subject. When the block

83a is moved to the position shown in Fig. 16B by manual manipulation of the handle 80, a vacuum is created into a cavity 90 between the bottom of the block and the base of the housing 82a. The spring 86 rapidly pushes the needle 83 through the orifice 82 and into the subject's skin. Blood from the subject due to the vacuum flows into the instrument along a passageway that includes the pellet 6 and the array EE. The filter 87 removes any cells and other particles from the sample (this is optional). The block 83a is then manually pushed back into the housing 82a using the lever 81 to first withdraw the needle from the subject. The excess blood will be absorbed by pad 88. The cavity 90 as shown by shaded areas is filled with this blood and any analytes in the blood interact with the binding molecules and enzymes on the surface of the electrodes in the array EE. The holding electrode 61, under the control of the microprocessor 82b, has its voltage regulated to collect any unbound particles 126. Any change in the electrical properties of the intersections between the electrode arrays EE are measured. A display for different analytes 91-95 on the exterior of the housing 82a provides both a qualitative and quantitative readout if any one, or all, of these analytes is present in the blood sample. The instrument I is designed to detect all five classes of analytes: Class I proteins, Class II nucleotides such as, for example, RNA and DNA, Class III small molecules, Class IV electrolytes, Class V cell detection. Bars, numbers, colors, text etc. are used to report the result. Fig. 16F depicts, for example, the display indicating that the sample was positive for the E-Coli and the level in the sample namely 10 units.

Eleventh Embodiment

The eleventh embodiment of this invention, the instrument J, shows a module 100a placed into an adapter 115a that is inserted into a USB port 190 of the personal computer shown in Fig. 20.

The module 100a is a hand held, disposable device similar in many respects to the instrument I, but designed to fit within the adapter 115a. It includes a needle 103 that is extended to collect a blood sample which flows past an array of electrode pairs, including the lower electrodes 100, 101, and 102 and an upper electrode 109 that is flexible and resilient similar to the electrode 64 used in the instrument G. The module 100a is shown with sample collected and the needle 103 withdrawn. Electrically readable particles 126 are shown adhering to the surface of the lower electrodes 100, 101, and 102 because of analytes present in the sample.

After collection of sample the module 100a is inserted into a adapter 115a that includes a roller 115 adapted to engage the electrode 109. The adapter 115a is compact, being shaped like a small box, so that it may be conveniently connected to the personal computer shown in Fig. 20. A drive mechanism 111a, including a screw 112 mounted between a pair of spaced supports 110 and 111 and a platform 114 support by another screw 113, serves to move the module 100a past the roller 115 and move the upper and lower electrodes from normally spaced apart positions into close proximity with each other sequentially like in the instrument G. A plug 117 makes an electrical connection with the computer through the electrical connectors 118 and 119 upon inserting the assembly of the adapter 115a and module 100a. Data that is gather corresponding to the presence or absence of analytes, and the amount, if any, present, is electronically transmitted, for example, over the internet, to insurance companies, a data base and other information services. This data may also be displayed on the monitor screen 190a of the computer.

Twelfth Embodiment

As depicted in Figs 21A through 21C and Fig. 22, the twelfth embodiment of this invention includes the instrument KA that is used with the detection system K. The system K automatically tests a series of sample containers 145 being moved in synchronization with a series of instruments KA. In the system K, a conveyor belt 140 moves the sample containers 145 past a rotatable collection member 140a having four radial arms 141-144. The collection member 140a and conveyor belt 140 are moved in a synchronous, step wise fashion, with each individual arm being positioned over each individual sample container 145 to draw the sample into an arm. A suction device (not shown) or other means is used to withdraw sample from a sample container 145.

Another conveyor belt 140a is moved in a synchronous, step-wise fashion with the movement of the belt 140 and collection member 140a. This belt 140 carries the series of instruments KA embodying this invention such as discussed above, preferably having an array of orthogonal electrodes 40-42 and 30-34 supported on substrates 121 and 120. A holding electrode 131 is used in the same manner as discussed above to add electrically readable particles 126 to the sample and collect unbound particles by controlling the voltage applied to this electrode. As shown in Fig. 22, the belts 140 and 140a and the collection member 140a move synchronously so that sample is collected by one arm 141 and then previously a collected sample in arm 143 is fed into an inlet port 130 of one instrument Ka. A cleaning station 148 is positioned at about 9 o'clock to sterilize the arms 141-144 after they deposit sample in the instrument KA advancing to a readout station 147a that detects changes in electrical properties due to the presence of analytes in the individual samples being tested. The readout station 147a includes a pressure application station 146 and a

detection circuit and signaling device as discussed herein contained within a housing 147.

With the step-wise movement of the belt 140a, the individual instruments KA are advanced past the station 146 at which a force is applied against the array of electrodes in each individual instrument KA to move the electrodes from a spaced apart position into close proximity with each. Qualitative and quantitative readouts are provided by the a detection circuit and signaling device if any analyte in a sample is detected.

Electrically Readable Particles

Electrically readable particles can be made of the same materials as electrodes. Types of electrically readable particles include metal spheres, nano and microcrystals, plastic particles containing paramagnetic or magnetic material, carbon nano-tubes, metal nano-wires, piezoelectric particles, and conductive plastic particles. They can be and are preferably coated with a mono- or multilayer. The outer layer should consist at least partially of the binding molecules. Gold particles are currently preferred, because of their stability in water, and constant size particles ranging from 1 nm to 3 μm are commercially available. Also various coating techniques for gold, such as self-assembled monolayers, have been developed. Electrically readable particles can be composites of several materials. Plastic particles can be coated by a metal layer. Again gold is the preferred material.

In accordance with one aspect of this invention, the electrically readable particles are coated, at least partially, with the binding agent (recognition molecules). These recognition molecules are often charged. For example, oligonucleotides are negatively charged in all common buffers. A certain protein is negatively charged, if pH is

above an isoelectric point and positively charged at lower pH. The charge is balanced by soluble counter ions, which form an electrical double layer. However, these particles move in an electric field electrophoretically. Also the particles repel each other because of ξ -potential. (J.N. Israelachvili "Intermolecular and Surface Forces", Academic Press, London, 1985).

Theory describes quantitatively the interaction charged colloidal particles. The charge of the electrically readable particles can be adjusted by attaching charged molecules, other than recognition molecules, onto the electrically readable particles. Examples of charged molecules are polylysine, polyasparagine acid, polyallylamine, polyacrylate, histone, and DNA. Oligonucleotides and DNA can be used to adjust the charge in immunoassays and also in DNA testing. In DNA tests the DNA that is used to regulate the charge must not contain a target sequence.

The charge may be regulated, for example, by pH, ionic strength, counter ions, solvent, and additives, such as polyethyleneglycol. Also the charged molecules may be attached on to the particles so that the binding is reversible. For example, if the surface of the particle is sparsely covered by negative moieties, such as carboxylates, an excess of a positive polymer, such as polyallylamine, will bind on to the surface and give the surface a positive charge. The negative charge of the carboxylates may be eliminated by adding acid so that pH is below 5. The binding of the positively charged polymer will be much weaker, and a significant part or possibly all of it will be detached from the particles. It is also possible to change the charge by a chemical reaction. For example, the positive charge of amino groups can be eliminated by acylation that is conveniently done in water by active esters, such as acyl N-hydroxysuccinimides.

Electrically readable particles may also be charged with static electricity, which may purposefully adjusted to be either positive or negative. The charge may also be changed during the assay. An additional charging electrode may be added to give the particles a charge before they enter into the assay area.

The charge of the particles can be utilized in many ways in assays. First, the particles may be forced to move through the sample electrophoretically, and while they are moving their recognition molecules will interact with the sample and collect analyte molecules. Second, the particles can be attracted on to the surface of one electrode. By alternating the charge of two electrodes that define one intersection, the particles can be concentrated on to that intersection. The binding kinetics of the particles will be much faster because of the concentration and the physical contact induced by the electric field. Third, by removing the particles by a reversed electric field, or by attracting the particles to another intersection, or by changing the charge of the particles, the strength of the analyte mediated binding can be measured. This is important to test the specificity of the binding. Specific and non-specific binding in immunoassays can be differentiated. Moreover, the binding force can be measured. In DNA tests this allows differentiation between SNPs (Single Nucleotide Polymorphisms). Fourth, the charge of the particle can be adjusted independently of an analyte. The charge can be even opposite to that of an analyte. For example, DNA binding particles may be made positively charged. After interacting with a sample these particles can be attracted on to the negative electrode surface. The soluble DNA in the sample as well as DNA that is weakly bound on to the particles would be repelled from the electrode.

Although the electric charge of an analyte has been used earlier to attract and remove the analyte from an electrode, the present

method is fundamentally different in several aspects. In this context the important difference is that the charge of the particle, and not the charge of an analyte, is the decisive factor. Also the whole electrode structure, and function as well as the detection method are significantly different from earlier approaches. All these aspects are discussed above in their appropriate context.

Electrodes

Although gold is currently preferred as an electrode material, many other conductive, semiconductive, magnetic, or piezoelectric materials are possible. Examples include platinum, palladium, osmium, iridium, silver, chromium, vanadium, tungsten, copper, nickel, graphite, semiconductors, such as silicon, germanium, zinc sulfide and selenide and conductive compounds and plastics, such as polyaniline, polyacetylene, polythiophene, and polyphenylene, tetrathiofulvalene, tetracyanoquinodimethane and their derivatives. Corrosion of an electrode prevents the use of some metals like iron and aluminum. Amalgams and composite materials are often more corrosion resistant, durable, and/or conductive than any pure component alone. Example is boron doped silver, several semiconductors, and carbon composites. If an electrode is coated by a thin protective layer, such as fatty acid monolayer, almost any metal can be used including iron and aluminum. This layer can be also conductive, for example, it can be gold, amorphous carbon, graphite, fullerene, tantalum nitride, tetrathiophene carboxylic acid, redox protein, such as cytochrome c, cytochrome c oxidase, or horse radish peroxidase. Electric current can flow across thin insulating layers by tunneling, and accordingly conductivity is not mandatory. Also when electrodes are brought into close proximity and compressed, the electrically readable particles can penetrate through a thin insulating layer. After the electrically

readable particles have been bound onto the electrode, it is possible to add a solvent that is able to remove the insulating layer, or enzymatically remove compounds such as carbohydrates, proteins, lipids, or nucleic acids.

The direct contact between the electrodes and electrically readable particles is not mandatory. Some electrical properties, for example capacitance, is modified by the presence of electrically readable particles between electrodes. Moreover, electric field is locally increased at the point in which electrically readable particles are located. Accordingly, chemical or electrical plating starts preferentially at these points. These plating methods can create an electrically conductive path between electrodes.

Electrode arrays are advantageously parallel stripes. By orienting two such arrays orthogonally, one with M and the other with N stripes, there are a total of $M \times N$ intersections (Fig. 12). The electrical properties of each intersection can be independently measured. These electrical properties include conductance, resistance, inductance, and capacitance. All of these can be correlated with the number of electrically readable particles in that specific intersection. The number of particles depends on the concentration of the analyte that is bound by the specific binding molecules in that intersection. Thus, the concentration of each of $M \times N$ analytes can be correlated with the electrical properties of the corresponding intersection.

Electrodes can be in shallow trenches. The depth of a trench should be about the same as the size of the electrically readable particle. Alternatively, electrodes can be separated by ridges that have about the same height as the diameter of the electrically readable particle. Instead or in addition to ridges, hillocks can be a part of the structure. The purpose of these structures is to prevent direct contact of two electrode surfaces. A third way to avoid the direct contact is to

use non-conductive particles as spacers. Also any combination of these methods can be used. spacers.

Still another method is to coat the counter electrode or electrode array with an essentially uniform layer of a soft material. Examples of such materials are silicone, vasoline, a mixture of triglycerides, and polyvinyl alcohol. When the electrodes are compressed into a close proximity, the spacer layer prevents direct contact between the electrodes, but the conductive particles will be able to penetrate through the spacer layer. This method has a further advantage that the space between the electrodes is essentially filled with a non-conductor.

Also any combination of the above methods may be used.

In a instrument having one roller (Figs. 14A and 14B) only one electrode needs to be patterned consisting of an array of $N^{1/2}$ electrodes for N analytes. On the other side is only one electrode that is about the same size as the whole counter electrode array. Registration can be maintained by controlling the position of the roller. It is also possible to add location electrodes into the array. These additional electrodes would contain location and other information, such as the type of the assay, in the form of the bar code that is read when a roller presses a counter electrode against them. The intersections are defined by the patterned electrodes and a roller. Only two electrodes are needed even for very large arrays with two orthogonal rollers, which in a preferred embodiment are orthogonal, but may be in any non-parallel orientation. The other roller would be under the lower substrate in Figs. 14A and 14B parallel to the surface of the paper. In the example depicted in Fig. Figs. 14A and 14B the roller presses one substrate. The electrode or electrode array can be directly on the surface of the roller. If two rollers are used, only one

electrode on each roller is needed even for millions of assays. The contact point between two rollers defines the assay site.

In another embodiment of the present invention, a conductive ball 73 forms a counter electrode (Figs. 15A, 15B, and 15C). This ball 73 should be at least ten times larger than the electrically readable particles 126 bound by affinity binding, and preferably they are macroscopic in the range of 0.1 mm to 50 mm, most advantageously between 1 mm and 5 mm. The contact point between the ball electrode 73 and the affinity electrode is the assay site. No patterning of the electrode is required, although patterning is still possible. Especially the surface can have a texture that controls the contact force and area.

In the preferred embodiments, the electrode is nonporous and flat. However, porous and textured electrodes have some merits. Grids, such as electron microscopy grids, allow a sample to flow through an electrode. This will increase the contact between the electrode and sample. Moreover, the electrodes are not necessarily rectangularly shaped. The areas containing binding agents (recognition molecules) may be enlarged or contracted. Also electrodes may be curved so that they diverge to allow easier contact with the outside macroscopic world.

The area of the intersection defined by two electrodes depends on the type and requirements of the assay. Qualitative assays may need only a very small area. It might be enough to bind ten electrically readable particles to achieve a reliable qualitative result. For a quantitative assay the minimum number of particles is about 100 otherwise the result is not reliable. For optimal accuracy the number particles should be about 10,000. This means that in a preferred embodiment the area of the intersection should be between 50 $\mu\text{m} \times 50$

μm and 500 μm × 500 μm. With the present invention it is possible to assay simultaneously 100 or even 10,000 analytes.

The present invention is in most embodiments a small instrument. It is possible to fabricate a really microscopic instrument that has the capability to measure multiple analytes. For example, microscopic forceps can be partially coated with metal. A part of this metal is further coated with binding molecules. An array of these modified forceps can perform multiple assays. The array can be inside a capillary tube, which can be so small that it can be used intravenously.

The instrument of the present invention can be manufactured by several means. In mass production, molding is a preferred method. Machining, laser, and water jet cutting may sometimes be advantageous. Figs. 11A through 11G depicts a photolithographic method, in which the second resist layer 59 defines wells 60 and also acts as a spacer. This method is amenable to small scale production. Methods that are better for mass production are given in Examples 1 and 2. Electrodes can also printed by screen-printing technology. For example, commercially available carbon ink paste and silver/silver chloride ink are suitable materials for working, counter, and reference electrodes. Other printing or stamping techniques, such as ink jet printing can be used.

The means for moving the electrodes from a spart apart to a close position included, but are not limited to, mechanical, hydraulic, electromagnetic devices. This movement may be induced by a spring, screw, liquid or gas pressure in a closed container, thermally induced expansion of the compressing part, solenoid, or piezo crystal (U. S. Patent No. 4,874,979) to mention the most important means. Moreover, compression may be achieved manually using a hand directly, or indirectly. Indirect compression may be induced by forcing

the instrument into a constrained space, in which the walls of the space force the capillary tube 641 to deform.

Attachment Of Recognition Molecules Onto The Electrodes

Binding agents, that is recognition molecules, can be bonded onto the surfaces by physical or chemical means. When a voltage, resistance, or current across an electrode intersection is to be measured, the electrically readable particles should be as close as possible to the electrodes. Accordingly, the recognition molecules should be either bonded directly onto the surface or connected with relatively short spacers. Electric current can easily flow over a thin monolayer by a tunneling mechanism. Longer spacers can be used, if they are electrically conductive. For instance, double helical oligonucleotides are electrically conductive and can be used as spacers. Compounds like tetrathiophene carboxylic acid are also conductive enough to be useful spacers. Small electrically readable particles, such as 1 - 100 nm gold spheres can be coated with recognition molecules and bound with the surface. The same or even better result can be achieved by having an electrode surface that has 1-100 nm roughness. Currently 10 - 20 nm surface roughness is preferred. A sputtered metal surface has typically this kind of roughness. Sharp edges and vertices are advantageous, because these will penetrate any spacer and layer of recognition molecules and create a direct electrical contact between the electrodes and the electrically readable particles.

Although metal surfaces are often polycrystalline, single crystal surfaces may sometimes be preferable. For instance, pyridine has highest affinity for a (210) gold surface of all possible gold surfaces, while it has the lowest affinity for a (111) gold surface. In general, positively charged surfaces have higher affinity for electrically neutral molecules than negatively charged surfaces. The problem with high

positive potential is that some important classes of compounds, such as mercapto compounds, will be oxidized and detached from the surface.

Spacers, recognition and other molecules can be bonded onto the surface by dispersion forces, hydrophobic force, hydrogen bonds, charge transfer, ionic or covalent bonds. Covalent bonds are strongest and most stable in hydrated milieu. Dispersion forces, such as van der Waals force, can be significant if a molecule is large and has tens of interactions, which combined can be comparable to a covalent bond.

Binding molecules can be adsorbed either from gaseous or liquid phase onto the surface. Laser ablation allows the evaporation of quite large molecules, such as peptides and oligonucleotides. However, proteins and oligonucleotides are preferably adsorbed from a buffer. Mercapto, amino, isonitrilo, carbonyl and carboxylate groups form bonds with various metals. Sulfur atoms interact especially strongly with gold and also with other noble metals. Organic mercapto compounds can also be dissolved into organic solvents, such as ethanol, and they will spontaneously form a self-assembled monolayer. Also ether oxygen binds with gold, although much more weakly than sulfur. Polymers containing many ether oxygens, such as polyethylene glycol will still bind strongly enough. Amphiphilic molecules form a monolayer at water-air interface. This monolayer can be compressed in a controlled way so that molecules occupy a desired average area. The monolayer can be deposited onto a solid surface such as an electrode.

After the recognition and other molecules have been deposited, they may be surrounded by a liquid, liquid crystalline, or solid matrix (the pellet 6, Fig. 16A) to increase their stability. The matrix is preferably such that it will be dissolved by a sample. It is also possible to add a special buffer or some other solvent before or during the assay to expose the recognition molecules. Well known stabilizers are,

for example, trehalose, glucose, glycine, glycerol, dextran, cyclodextran, starch, polyvinyl alcohol, and polyethylene glycol. Surfactants may be added to speed up the hydration. Surfactants include tween-20, octyl glucoside, sodium dodecyl sulfate, sodium palmitate, potassium oleate, sodium cholate, trimethyl octadecyl ammonium bromide, and phospholipids, such as dihexanoyl phosphatidyl choline, 1-palmitoyl-2-oleoyl-sn-3-phosphatidyl choline, and 1,2-dilinolenoyl-sn-3-phosphatidyl ethanolamine. Various salts, such as sodium chloride, sodium phosphate, sodium acetate, potassium lactate, sodium citrate, calcium chloride, and magnesium chloride, may be a part of the formulation. Combinations of these and other compounds can be used to reduce crystallization. These compounds are often solubilized into water, and the water solution is applied by a pipette, ink jet printer, or by pins on to the surface. Water is best removed by lyophilization.

Reagent patterning and stabilization for such testing are discussed in the following references: United States Patent Nos. 3,572,400; 4634027; 4216245; 5001048; 5554339; 4329317; and 5413732; and J. Lipkowski and P.N. Ross, "Adsorption of Molecules at Metal Electrodes", VCH, New York, 1992.

Oligonucleotides may be terminated by amino, mercapto, biotinyl, and several other groups. There may be several amino or other groups as well as combinations of various groups. To give more freedom for oligonucleotides these groups, which are intended to bind with a substrate, can be separated from the actual oligonucleotide sequence with a spacer. Spacer is preferably water soluble polymer, such as polyethylene glycol, polyvinyl alcohol, polyacrylic acid, or optionally a copolymer of two or more hydrophilic and hydrophobic monomers.

Ionic and Electronic Current

Immediately after a potential is coupled over an electrode intersection that has electrically readable particles, there will both ionic and electronic current. The Nernst-Planck equation gives the flux of any ionic or molecular species in the electric field.

$$J_i(x,t) = -D_i \frac{fC_i(x,t)}{fx} - \frac{zFD_i fC_i(x,t)}{RT} + V(x,t)C_i(x,t)$$

Each ionic species may have different diffusion constant D_i , charge z_i , and concentration $C_i(x,t)$. Each has an effect on the potential $\phi(x,t)$. Thus, an analytic solution is in most cases very difficult or even impossible and the equations must be solved numerically using a computer. The diffusion coefficient is usually between 10^{-5} and 10^{-6} cm^2/s . This means that the molecules and ions will diffuse from one electrode to another in a fraction of a second, because of very small distance between the electrodes, that is, no greater than 1000 nanometers. The electric field induced transport is faster for any significant potentials. Based on this qualitative inspection, it is possible to deduct that the ionic current will last only a very short time and an equilibrium is established. This corresponds to a charging of a capacitor. After charging, no ionic current should flow, provided that no electrochemical reactions are happening on the electrode surfaces.

After the electrodes are compressed into a close proximity, the volume between the electrodes is so small that electrochemical reactions consume the electroactive compound very fast. Accordingly, the measurement must also be fast. While this might first seem to be a drawback, it is really a merit. The behavior of the ions depends on the reversibility of the oxidation or reduction much more than in a large

traditional electrochemical cell. For a reversible redox reaction the reduced species can diffuse from the cathode to the anode very fast and be reoxidized and then diffuse to the cathode and be reduced again. For an ideally reversible redox reaction the cycle can be repeated without any limit. This leads to a greatly amplified signal. For an irreversible reaction, a gradual increase of an electric potential will deplete the compounds having lowest oxidation or reduction potentials. When the potential increases they do not interfere with the signal obtained from the compounds having higher oxidation or reduction potentials. This is in contrast with bulk electrochemical methods, in which compounds having lower potential continue to give signal during the potential sweep even if the reaction is irreversible.

Referring to Fig. 17, the resistance R of a conductive sphere 4 having a radius r is

$$R = \frac{\rho}{\pi r} \ln \frac{r+a}{r-a}$$

where a is the distance of the contact area from the center of the sphere. The resistance of a gold sphere that has a radius of 200 nm, and is embedded about 5 nm into a conductive layers on both sides, is about 0.2 Ω . Gold electrodes that have dimensions of 100 nm x 1 mm x 10 mm have a resistance of 2 Ω . If the particle is coated with a conductive layer of a thickness b , the resistance is

$$R = \frac{\rho a}{\pi b r}$$

The current between electrodes that are connected by N particles is

$$I = \frac{\rho a U}{\pi r b N}$$

This is a big enough a current to be measured by hand held amperometers. If there is an organic mono- or multilayer between the gold sphere and the electrode, the current must propagate by tunneling. The tunneling current will be only few nanoamperes via one gold particle. Because there are ideally thousands of gold particles in one assay area, the current will be typically micro- or milliamperes.

In some situations, the conductivity of the gold particle is reduced by applying as resistive material as a layer b over the surface of the gold particle as depicted in Fig. 17.

Immunoassays

Antibody-antigen interaction can be used in many different ways either to bind or prevent the binding of electrically readable particles onto a surface of an electrode. In a traditional sandwich type assay one member of a matching antibody pair is bound on to electrically readable particles and the other on to an electrode. The corresponding antigen will form a bridge between two antibodies and bind the electrically readable particle on to the surface of the electrode as is depicted in Figs. 1A and 3B. This is a preferred approach when an antigen is a large enough molecule to have at least two spatially separated epitopes. Most proteins can be assayed by sandwich assay. A cleavable spacer provides another way to perform a sandwich assay (Virtanen, 1996). The number of the bound electrically readable particles is directly proportional to the concentration of the antigen. For smaller molecules, such as steroids and several drugs, the competitive assay is the method of choice (Figs. 3A and 3B). The antibody is attached, for example, on to the electrically readable

particle, and the antigen or an analog of the antigen is attached onto the electrode. The antigens in the sample saturate the antibody molecules on the electrically readable particles in a concentration dependent manner. The competitive method is not equally sensitive as the sandwich assay, because zero and very small concentrations of the antigen gives maximum or near maximum binding. Small changes are very difficult to differentiate from the normal experimental error.

Currently the preferred electrode material is gold. The gold surface can be first coated with streptavidin, which will adsorb spontaneously from an aqueous solution. Biotinylated antibody will bind with streptavidin providing a good coating. Several other ways of attaching antibodies onto the gold surface are well known in the art. These include forming a monolayer of polylysine or copolymer of lysine and cysteine on to the gold surface, and attaching periodate oxidized antibody in the presence of sodiumcyanoborohydride on to this monolayer. The simplest way is to reduce the antibody with dithiotreitol or with some other reductant and let the reduced antibody to chemisorb directly on to the gold surface. This is currently the preferred method, because the insulating organic layer is very thin, having a thickness of only half of an antibody molecule. The preferred electrically readable particles of gold spheres can be coated with antibodies using exactly the same methods as are used to coat the gold electrode. A wide variety of immunoassays can be performed with the present method. Nonlimiting examples include HCG (pregnancy test) and prostate specific antigen (PSA) detection, insulin, proinsulin, glucagon, glycated hemoglobin, growth hormone, fetoprotein, TSH, C-reactive protein, CK-MB, myoglobin, troponin, interferons, interleukins, ferritin, tumor necrosis factor, trypsin, plasminogen, cardiolipin, cortisol, aldosterone, estradiol, digoxin, benzodiazepine, vancomycin, amphetamine, cocaine, morphine, tetrahydrocannabinol,

phenobarbital, secobarbital, parathione, adenovirus, chlamydia, cytomegalovirus, hepatitis viruses, HIV, influenza, and parainfluenza.

Immunoassays and panels are discussed in the following United States Patent Nos. 5,744,358; 5,075,220; 5,030,561; 4,497,900; 4497899; and in Kohler et al., "Antigen Detection to Diagnose Bacterial Infections", Boca Raton, Florida, CRC Press, Inc. 1986, pp. 138-144.

DNA testing.

DNA tests are analogous to sandwich immunoassays as is depicted in Figs. 2A and 2B. The present invention allows testing of tens or even hundreds of thousands of sequences simultaneously. The main large-scale application of oligonucleotide arrays is comparative expression analysis. Gene expression patterns in healthy as well as in diseased tissues and cells will greatly increase the understanding of the function of living organisms. The effect of drugs can be understood in much more detail than presently. Another application for the oligonucleotide arrays is the finding of single nucleotide polymorphisms (SNPs). It is estimated that in the human genome one out of a thousand nucleotides is polymorphic. These SNPs are the main reason for human diversity. Once the SNPs have been characterized and correlated with certain disease states, SNPs can be used to predict an individual's tendency for many diseases including various cancers (Cole et al. (1999) The genetics of cancer-3D model, *Nature Genetics Supplement* 21: 38-41), heart disease (Lusis (2000) Atherosclerosis, *Nature* 407: 233-241), and Alzheimer's disease. Another example of DNA diagnostics is the detection of fetal DNA in maternal plasma (Lo (2000) *Clinical Chemistry* 46:1903-1906). These DNA and RNA studies are not limited to humans. Plant genomics have enormous economical importance. Knowledge of pathogen genes and gene expression can be used in diagnostics and for the design of new drugs.

In DNA testing the binding agent is usually called a probe and the analyte is usually called a target. Oligonucleotide probes can be synthesized so that they contain aliphatic amino, mercapto, or other groups. They will bind spontaneously on to an electrode surface. Mercapto groups have a drawback that they tend to be oxidized by the positive electrode (anode). The positive charge is sometimes used to attract sample DNA to the close proximity of the electrode. According to the present invention, particles and not the analytes themselves are attracted to the electrode. The charge of the particles can be opposite to the charge of an analyte. Even, when particles are partially covered by negatively charged oligonucleotide probes, which further bind negatively charged targets, the particles can be positively charged or electrically neutral. Thus, it is possible to use a negative charge to attract these particles. In addition to stabilizing the sulfur-gold bond, the negative charge repels the oligonucleotide probes on the surface of the anode. The probes will be nearly perpendicular to the surface and easily accessible (Figs. 2A and 2B). In the case when positive charge is used, the probes are likely to lay flat on the surface being sterically hindered. The probes have 5 to 10,000 nucleotides, and preferably 10 - 80 nucleotides. Long probes have generally genomic origin. The target is often a PCR product (amplicons). Other amplification methods are equally possible, including isothermal and ligation amplification. The probes should have preferably about the same length as the amplicons.

Hybridization conditions are well known in the art. Temperature should preferably be 20 °C below the melting temperature of the duplex. The salt concentration has a very large effect on the melting temperature and kinetics of the hybridization. The melting temperature depends also on the guanine and cytosine contents of the probes and the target. A higher salt concentration in the buffer and higher C/G-content will increase the melting temperature. The

electric current will increase the temperature of the electrolyte. The power input is $P=UI$, where U is the potential and I is the current. The increase in the temperature is $\Delta T=UIT/Cm$, where t is the time and C is the thermal capacity of the medium of a mass m . A certain thin layer of the walls must be included into the mass m . The method of the present invention is less sensitive to the external conditions than most currently used methods. This is due to the electric field that can be utilized to increase the rate of the hybridization as well as to test the stringency of the hybridization.

Polymerase and ligase chain reactions as well as isothermal amplification can be performed in the device of this invention. The temperature cycling (not for isothermal) can be obtained by electric potential induced heating between the electrodes or by embedding heating elements near the electrochemical cells. The heating is more effective, if instead of a direct current, an alternating current is used.

Oligonucleotide analogs, such as peptidenucleotide acids (PNAs), and thionucleotide acids, offer often increased stability and/or stringency of hybridization. Oligonucleotide probes may be prehybridized with complementary oligonucleotides. The purpose of this kind of array is to study interaction of biomolecules with double helical DNA.

Electrolytes

As discussed above, the detection of electrolytes is one feature of the present invention, because electrolytes are commonly measured electrochemically. Although electrolytes are most of the time measured via the potential change they cause, the present invention offers some advantage over the traditional electrochemical methods. Bringing the electrodes into close proximity will reduce or eliminate the non-specific ionic background current. A steady state ionic

distribution is established very fast, generally in the order of seconds or less. The subsequent current will be due to specific binding or permeation of ions.

Solid state electrodes are currently preferred, because they are easier to store than electrodes containing liquids. Ionic specificity is obtained either by superionic films or by ion selective membranes. Advantageously, a reference electrode is used in addition to working and counter electrodes.

Electrolyte detection is discussed in the following references: United States Patent Nos. 5401378; 4758325; 4973394; 5234566; and in D. Ammann, "Ion-Selective Microelectrodes: Principles, Design, and Application", Springer-Verlag, New York, 1986, and A.L. Laskar and S Chandra, "Superionic Solids and Solid Electrolytes. Recent Trends", Academic Press, Inc., New York, 1989.

Small Molecule Assays

As discussed above, the present invention allows the detection of several small molecules including, but not limited to glucose, ethanol, cholesterol, lactic acid, and bilirubin. Conventional techniques for binding are discussed by Wieck et al. (1984) *Anal. Chim. Acta* 158: 137. These small molecules do not in most cases have any specific binding molecule. Instead, each of these compounds has a specific oxidizing or reducing enzyme. Oxidizing enzymes often consume oxygen for oxidation. Oxygen is reduced typically into hydrogen peroxide instead of water. Hydrogen peroxide can be detected electrochemically that is natural detection method for the present invention. Alternatively hydrogen peroxide can enzymatically oxidize many compounds, such as benzidine, tetramethyl benzidine, p-fluorophenol, or p-fluoroaniline to produce fluoride ion, or some other

a secondary stable species that can be detected by an ion selective electrode.

Cell and Pathogen Detection

Cells and pathogens can be detected either by assaying certain surface markers, or their DNA. The methods already described for immunoassays and DNA testing are applicable. The conductive particles can also be bound onto the cell membrane, either before or after being bound onto the surface of an electrode. When electrodes are compressed, the conductive particles are forced into a contact with the electrodes. Examples of cells include the human T&NK cells (CD2 and CD7), T helper cells (CD4), T cells (CD5), suppressor cells (CD8), E. Coli, Salmonelle, and Helicobacter pylori.

Detection Circuits

Typical detection circuits are illustrated in Figs. 23, 24 and 25.

In the detection circuit X shown in Fig. 23, the electrodes 25 and 30 are on one leg of a conventional Wheatstone bridge circuit 144a having AC current applied across this circuit from the source 140. Resistors 144, 145, and 146 are in the other legs of this circuit 144a. An oscillator 148 provides a signal that varies depending on the electrical characteristics or properties of inductance, resistance, capacitance and/or phase shift that is detected by a phase shift detection circuit 145a.

In the detection circuit Y shown in Fig. 24, the electrodes 25 and 30 are in a DC circuit where an input voltage V_i is applied across a capacitor 155 in parallel connection with the electrode 25 and 30. A charging pulse is applied to the base of a transistor 148 and this transistor's emitter is connected through the diode 160 to the + input of an operational amplifier 149, with a feed back loop 149a connecting

the amplifier's output to the - input. The output of the amplifier 149 is applied across a voltage measuring device Vmeas to ground. The voltage measured by the voltage measuring device Vmeas varies depending on the electrical characteristics or properties of resistance and/or capacitance across the electrodes 25 and 30.

The detection circuit Z shown in Fig. 25 is like that shown in Fig. 24 , but it is designed to be used with an array of test sites such as depicted in Fig. 9. It additionally includes a multiplexer 165 for connecting the test sites 150, 151, and 152, each including the pair of electrodes 25 and 30, the separate circuits including the diodes 160, 161 and 162, respectively, to the + input of the amplifier 149.

Measurement Of The Electrical Properties

Electrical power may be provided by a battery (Galvanic cell), solar cell, electromagnetic radiation, magnetic induction, direct contact with external power source, or by any other commonly known means.

Almost any electrical measurement instrument that is able to measure either voltage, current, capacitance, inductance, impedance, or phase shift can be a part of the present invention (A.J. Bard and L.R. Faulkner "Electrochemical Methods: Fundamentals and Applications", Wiley, New York, 1980). The coupling of these instruments to the processing units and to the networks is well known in the art. Examples can be found in several books, including J.J. Barbarelllo "PC Hardware Projects, Volume 3" (Prompt Publications, Indianapolis, 1998), W.J. Tompkins and J.G. Webster "Interfacing Sensors to the IBM PC" (Prentice Hall PTR, Englewood Cliffs, 1998), S. McDowell and M.D. Seyer "USB Explained" (Prentice Hall, Upper Saddle River, 1999), and J. Axelson "Serial Port Complete" (Lakeview Research, Madison, 1998). Networks include Local-Area-Networks, and Internet. Networks can

utilize among others, metal cables, fiberoptics, or be wireless. The ComponentLab described by Barbarello is especially well suited for the measurement of the electrical properties of the electrode intersections. This instrument is totally controlled and powered by a PC. All electrode intersections can be probed sequentially. The unit also contains an A/D converter. Voltage, resistance, and capacitance can be measured for each intersection, and results can be displayed either in a table or graphical format.

The direct detection of the tunneling current is possible, but the charge-and-leak circuit of Fig.24, called Charge-and-Leak Amplifier, is one option. In the case of a direct contact between the gold particles and electrodes, the current can be inconveniently big, unless resistors are added into series with the gold particles. A Sample-and-Hold Amplifier (J.J. Brophy, Basic Electronics for Scientists, McGraw-Hill Publishing Co, New York, 1990, p. 234) can be modified so that it can be applied to measure electrical properties of each electrode intersection. Input voltage is constant voltage source. A short pulse, for example 50 μ s, is given to the base of a transistor 87. A very low leakage capacitor 85 is charged via a diode 90. In parallel with this capacitor is the electrode intersection that is a leaking capacitor 80, if electrically readable particles are bound between the electrodes. The voltage is measured through a high impedance Op-Amp 89 that contains field-effect transistors. Without the measurement cell the capacitor would retain over 99% of its charge more than 100 hours. The leakage that is due to the cell will reduce the charge and voltage exponentially. This reduction can be sampled at desired intervals or continuously. The time constant of the leakage can be correlated with the number of electrically readable particles.

EXAMPLES

The following are examples of making the electrodes used in this invention.

Example 1

A 4 inch or 6 inch silicon wafer is spin coated with a resist layer. After photolithography 200 nm of silicon is etched away from the exposed areas (Hsiao, Virtanen, and Penner (1993) Appl. Phys. Lett. 63: 1119-1121) to produce trenches. On to these trenches electrodes will be formed by evaporation or sputtering through a mask. Alternatively, electrodes are made by photolithography. The electrode pattern is clearly visible at this point. The wafer is covered by a resist layer. The disc through a mask is exposed to UV-light to form the assay sites. After washing, the assay sites on the electrode array are uncovered gold surrounded by a resist layer. In order to remove any residue, the whole disc is treated with oxygen plasma. Thiolated oligonucleotides and antibodies are printed by ink jet printer, solenoid printer, or by pins (not to be confused with the electrical pins that provide the contact to external instruments). If assay sites are large enough, a pipetting machine will be used to dispense the oligonucleotide probes. In this example fifteen different recognition molecules are dispensed.

Example 2

An electrode array can be conveniently fabricated starting from a commercially available unprocessed CD-master that is a glass plate coated with photoresist. The thickness of the photoresist is about 200 nm that is ideal for most implementations of the present invention. The CD-master is irradiated by UV-light through a master. The exposed

areas are dissolved away. A metal film, for example a nickel film, is evaporated evenly over the patterned master disc. After electroforming the metal is detached and used like a CD-stamper is used in CD production. Molded parts are coated with a metal layer either by sputtering or evaporation. The currently preferred metal is gold. A mask is used to allow the metal to be deposited only on to electrode areas. Chemicals are dispensed as in Example 1.

Example 3

Into 1 ml of 200 nm gold colloid (Ted Pella, Inc.) is dialyzed against PBS. Simultaneously into 100 μ l of 1 μ M solution of polyallylamine is added 10 μ l of 1 mM SPD in PBS in another dialysis tube. After 8 hour dialysis 10 μ l of 1 μ M solution of polyallylamine in PBS is added into the dialysis tube containing gold colloid. The dialysis is continued 16 hours, and 10 μ l of 1 mM solution of SMMM is added. The dialysis is continued 8 hours. Simultaneously anti-human IgG is reduced with dithiotreitol and dialyzed against PBS buffer under nitrogen atmosphere. 100 μ l of the antibody solution is added into the gold colloid dialysis tube, and the dialysis is continued another 8 hours under nitrogen atmosphere. The dialysis buffer is changed twice during each dialysis.

Example 4

Each of the fifteen assay sites of an electrode array (Examples 1 and 2) is coated with 10 nl of reduced antibody solution (Example 3). In this example each assay site is coated with the same antibody. However, in a preferred embodiment different recognition molecules are dispensed to each assay site excluding sites that are reserved for calibration,

which have duplicates or even multiple repeats. After 30 min the array is washed with PBS. 5 nl of 1 M trehalose solution containing also 0.5% of tween-20 is dispensed by ink-jet printer on to each assay site and the array is lyophilized to preserve the antibodies. In this specific example a dilution series of human IgG is studied by the electrode array. Three of the assay sites are reserved for controls, and the other 12 are used for duplicate measurements for six samples of the dilution series. The gold particles used in this example have a positive charge even above isoelectric point of the antibodies that are attached on to them. The affinity bonding can be sped by applying a negative potential to the assay sites. Non-specifically bonded particles can be removed by reversing the potential before the electrodes are compressed to a close proximity. The current, voltage, inductance, capacitance, and/or some other electrical property of each electrode intersection is measured.

SCOPE OF THE INVENTION

The above presents a description of the best mode contemplated of carrying out the present invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains to make and use this invention. This invention is, however, susceptible to modifications and alternate constructions from that discussed above which are fully equivalent. Consequently, it is not the intention to limit this invention to the particular embodiments disclosed. On the contrary, the intention is to cover all modifications and alternate constructions coming within the spirit and scope of the invention as generally expressed by the following claims, which particularly point out and distinctly claim the subject matter of the invention:

THE CLAIMS

1. An instrument for detecting in a sample the presence of an analyte, said sample, having introduced therein prior to, during, or after the sample is fed into the instrument, electrically readable particles with an agent attached thereto that binds with the analyte, or the agent on the particles is an analog of the analyte and does not bind to the analyte,

 said instrument including

 a port through which the sample is fed into the instrument,
 first and second electrodes, said first electrode including a surface portion having an agent thereat that binds with the analyte, or said agent on the surface portion is an analog of the analyte and does not bind to the analyte,

 said electrodes having a first position where they are separated a first distance apart sufficient to enable the sample to move between said electrodes and a second position where the electrodes are in close proximity to each other, said first distance being greater than said second distance,

 a detection circuit, including the electrodes, that has a first state when the analyte is absent from the sample and a second state when the analyte is present in the sample, said second position determining the state of the detection circuit,

 a signaling device that provides an indication of the state of the detection circuit with the electrodes in the second position.

2. The instrument of Claim 1 wherein pressure is applied against any particle or particles bound to the surface portion of said first electrode.

3. The instrument of Claim 1 wherein the electrically readable particles have a diameter of from 1 nanometer to 1 millimeter to enable the instrument to detect in the sample a single molecule of analyte.
4. The instrument of Claim 1 where the particles are selected from a group consisting of metals and plastics.
5. The instrument of Claim 4 where the particles are essentially entirely of gold.
6. The instrument of Claim 4 where the particles have an exterior surface of gold and an interior core of an insulator material.
7. The instrument of Claim 4 where the particles have an exterior surface made of an insulator material and an interior core of gold.
8. The instrument of Claim 4 where the particles include a ferrous metal.
9. The instrument of Claim 1 including a system that removes particles that are not bound to the first electrode from between the electrodes prior to operation of the detection circuit.
10. The instrument of Claim 1 where the signaling device provides a qualitative read-out identifying the presence of the analyte or a quantitative read-out corresponding to the amount of analyte in the sample.

11. The instrument of Claim 1 where the signaling device provides a qualitative read-out identifying the presence of the analyte and a quantitative read-out corresponding to the amount of analyte in the sample.
12. The instrument of Claim 1 where the first and second states correspond to differences in conductivity.
13. The instrument of Claim 1 where the first and second states correspond to differences in inductance.
14. The instrument of Claim 1 where the first and second states correspond to differences in capacitance.
15. The instrument of Claim 1 where the first and second states correspond to differences in voltage.
16. The instrument of Claim 1 where the first and second states correspond to a phase change.
17. An instrument for detecting in a sample the presence of an analyte having individual molecules with at least two spaced binding sites, said sample, having introduced therein prior to, during, or after the sample is fed into the instrument, electrically readable particles with an agent attached thereto that binds to one of the binding sites, said instrument including
a port through which the sample is fed into the instrument,
first and second electrodes, said first electrode including a surface portion having an agent thereat that binds with the binding site of the analyte not bound to an electrically readable particle,

said electrodes having a first position where they are separated a first distance apart sufficient to enable the sample to move between said electrodes and a second position where the electrodes are in close proximity to each other, said first distance being greater than said second distance,

a detection circuit, including the electrodes, that has a first state when the analyte is absent from the sample and a second state when the analyte is present in the sample, said second position determining the state of the detection circuit,

a signaling device that provides an indication of the state of the detection circuit with the electrodes in the second position.

18. The instrument of Claim 17 wherein pressure is applied against any particle or particles bound to the surface portion of said first electrode.

19. The instrument of Claim 17 where the agent on the particle and the agent on the electrode are different.

20. An instrument for detecting in a sample the presence of an analyte, said sample, having introduced therein prior to, during, or after the sample is fed into the instrument, electrically readable particles with an agent attached thereto that binds to said analyte,
said instrument including

a port through which the sample is fed into the instrument,
first and second electrodes, said first electrode including a surface portion having thereat an analog of the analyte,

said electrodes having a first position where they are separated a first distance apart sufficient to enable the sample to move between said electrodes and a second position where the electrodes are in close

proximity to each other, said first distance being greater than said second distance,

a detection circuit, including the first and second electrodes, that has at least one electrical property of the circuit that (a) does not change because the analyte is present in the sample and attaches to the agent on the electrically readable particles and (b) that changes in the absence of the analyte due to the agent on the electrically readable particle binding to the analog of the analyte on the electrode, and

a signaling device that provides an indication when analyte is not present or present.

21. The instrument of Claim 20 wherein pressure is applied against any particle or particles bound to the surface portion of said first electrode.

22. The instrument of Claim 20 where the agent on the particle and the agent on the electrode are different.

23. An instrument for detecting in a sample the presence of an analyte, said sample, having introduced therein prior to, during, or after the sample is fed into the instrument, electrically readable particles with an agent attached thereto that binds with the analyte,

said instrument including

a port through which the sample is fed into a chamber including a first electrode and a second electrode including a surface portion having thereat an agent that binds with the analyte,

a detection circuit, including the first and second electrodes,

at least one of said electrodes being connected to drive system that moves said electrode between a first position where the detection circuit has a first state and a second position where the detection

circuit has a second state when the analyte is present in the sample and attaches to the agent on the electrically readable particles and the agent on the electrode, and

a signaling device that provides an indication of the presence or absence of analyte with the electrodes in the second position.

24. The instrument of Claim 23 wherein pressure is applied against any particle or particles bound to the surface portion of said first electrode.

25. The instrument of Claim 22 where the agent on the particle and the agent on the electrode are different.

26. An instrument for detecting and identifying multiple analytes in a sample, said sample, having introduced therein prior to, during, or after the sample is fed into the instrument, electrically readable particles, said particles having different classes of binding agents thereon, each class binding with only one of the multiple analytes,

said instrument including

a port through which the sample is fed past a plurality of assay sites formed by pairs of intersecting electrodes that are components of a detection circuit,

each pair of intersecting electrodes including one electrode with a surface portion having thereat a binding agent that binds with only one specific analyte to make each assay site specific to said one analyte,

each said assay site having a first state in the absent of the analyte specific to a site in question and a second state when the analyte specific to the site in question is present and attaches to the

binding agent on the electrically readable particles that attach only to the assay site in question,

said detection circuit including a signaling device that provides an indication for each assay site when analyte is present or absent at an assay site.

27. The instrument of Claim 26 when at least one of each pair of electrodes at each assay site is moveable.

28. The instrument of Claim 26 including a system that removes particles that are not bound to an electrode at an assay site from between pairs of intersecting electrodes prior to operation of the detection circuit.

29. The instrument of Claim 26 where the signaling device provides a qualitative read-out identifying the presence of the analyte or a quantitative read-out corresponding to the amount of analyte in the sample.

30. The instrument of Claim 26 wherein pressure is applied against any particle or particles bound to the surface portion of said first electrode.

31. The instrument of Claim 26 where the signaling device provides a qualitative read-out identifying the presence of the analyte and a quantitative read-out corresponding to the amount of analyte in the sample.

32. A portable, single use instrument for detecting in a sample the presence of an analyte,

 said instrument including

 a housing having a size that allows the instrument to be held by a user,

 said housing having a passageway therein with a port through which the sample is fed into a testing chamber,

 a supply of electrically readable particles with an agent attached thereto that binds with the analyte, said electrically readable particles being introduced into the sample,

 a sample collection device including a needle mounted within the housing in advance of the chamber and moveable outward from the housing to make contact with and draw the sample from a host and, after drawing the sample, moveable to within the housing, to feed the sample into the testing chamber,

 a pair of electrodes at the chamber, one having a surface portion with an agent thereon that binds with the analyte,

 a detection circuit, including the pair of electrodes,

 at least one of said electrodes being moveable between a first position where the detection circuit has a first state and a second position where the detection circuit has a second state when the analyte is present in the sample and attaches to the agent on the electrically readable particles and the agent on the electrode.

33. The instrument of Claim 32 including a receptacle along the passageway in advance of the testing chamber holding the supply of electrically readable particles.

34. The instrument of Claim 32 where the receptacle includes a solid member comprising a matrix material holding the particles, said matrix

material dissolving in the sample as said sample flows along the passageway past said solid member to release the particles.

35. The instrument of Claim 32 where the sample collection device collects a sample having a volume that does not exceed 5 milliliters.

36. The instrument of 35 where the sample collection device collects a sample having a volume that is from 1 picoliter to 5 milliliters.

37. An instrument for detecting in a sample the presence of multiple classes of analytes,

said instrument including

a grid of assay sites, an electrical property at each site being a function of the absence or present of a specific analyte for a specific class of analytes,

each site including a pair of electrodes at least one being moveable, said electrodes initially spaced apart a distance from 5 nanometers to 10 micrometers, with each pair of electrodes oriented at a right angle with respect to each other,

a detection circuit including each pair of electrodes to produce a change in the electrical property at each individual site whenever the absence or presence of a specific analyte for a specific class of analytes at each site is detected, and

a signaling device providing a signal whenever a response is detected at a specific site by the detection circuit, said signal being specific to identify the site where the response is detected.

38. The instrument of Claim 37 where the electrodes formed by a photo-resist etching process.

39. The instrument of Claim 37 where the electrodes formed by a plating process.

40. The instrument of Claim 37 where the signal provides a qualitative read-out identifying the presence of the analyte detected at each individual site or a quantitative read-out corresponding to the amount of analyte detected at each individual site.

41. The instrument of Claim 37 wherein pressure is applied against any particle or particles bound to the surface portion of an electrode, said pressure being not so great as to cause a short circuit between the electrodes.

42. The instrument of Claim 37 including a system that removes particles that are not bound to an electrode at an assay site from between pairs of intersecting electrodes prior to operation of the detection circuit movement.

43. An instrument for detecting in a sample the presence an analyte including

a disposable sample collection member removably connected to a receiving module adapted to be connected to a computer to which test data derived from sample collection member is transmitted,

said sample collection member comprising a housing holding a needle mounted to be extended after contact with the subject to withdraw a blood sample from the subject and then be retracted within the housing to introduce the sample into a test chamber including an electrode that is sensitive to an analyte in the sample, the presence of said analyte altering an electrical property of a detection circuit, said

detection circuit providing an indication when the analyte is detected or absent.

44. A method of taking, analyzing and reporting results of a blood test after taking a sample from a subject, including the steps of providing a portable instrument that is held by a user and includes a syringe needle that is contained within a housing so that the user is protected against accidental needle sticks and is extended after firm contact with the subject to pierce the skin and withdraw a blood sample from the subject and then retracted within the housing to introduce the sample into a test chamber including an electrode that is sensitive to an analyte in the sample, the presence of said analyte altering an electrical property of a detection circuit, said detection circuit providing an indication whether the analyte is detected or absent,

while holding the instrument, bringing the instrument into contact with the subject and extending the needle to withdraw blood from the subject, and

retracting the needle within the instrument and depositing the sample in the test chamber where the presence or absence of the analyte is detected by the detection circuit and displayed to report the results.

45. The method of Claim 44 wherein said method is conducted within less than 1 hour.

46. The method of Claim 41 where the display of results provides a qualitative read-out identifying the presence of the analyte and a quantitative read-out corresponding to the amount of analyte in the sample.

47. A method of detecting in a sample the presence of an analyte, including the steps of

(a) introducing into the sample electrically readable particles with an agent attached thereto that binds with the analyte or is an analog of the analyte,

(b) providing an instrument including

a pair of electrodes, said electrodes having a first position where they are separated a first distance apart sufficient to enable the sample to move between said electrodes and a second position where the electrodes are in close proximity to each other, said first distance being greater than said second distance,

a detection circuit, including the electrodes, that has a first state when the analyte is absent from the sample and a second state when the analyte is present in the sample, said second position determining the state of the detection circuit,

a signaling device that provides an indication of the state of the detection circuit with the electrodes in the second position,

(c) contacting the sample, including the electrically readable particles, with the electrodes while said electrodes are in the first position,

(d) removing any unbound particles from between the electrodes, and

(e) moving the electrodes to the second position.

48. The method of Claim 47 where the electrically readable particles have a diameter of from 1 nanometer to 1 millimeter.

49. The method of Claim 47 wherein pressure is applied to the electrodes while in the second position.

50. The method of Claim 47 where the particles are selected from a group consisting of metals and plastics.

51. The method of Claim 50 where the particles are essentially entirely of gold.

52. The method of Claim 50 where the particles have an exterior surface of gold and an interior core of an insulator material.

53. The method of Claim 50 where the particles have an exterior surface made of an insulator material and an interior core of gold.

54. The method of Claim 50 where the particles include a ferrous metal.

55. The method of Claim 47 including a system that removes particles that are not bound to the electrode from the vicinity of the electrode prior to operation of the detection circuit.

56. A method of detecting in a sample the presence or absence of an analyte including the steps of

(a) mixing with the sample electrically readable particles having thereon a binding agent,

(b) passing the sample, including said electrically readable particles, past a pair of moveable electrodes, one of which has a binding agent thereon, said electrodes being connected to a detection circuit with at least one electrical property that is affected or not affected when the analyte is present and binds or fails to bind with the agent on the electrically readable particles , and

(c) monitoring said electrical property of the detection circuit as the sample passes by the electrode to detect whether said electrical property is affected or is not affected.

57. The method of Claim 56 where the particles have a gold core at least partially coated with an insulator.

58. The method of Claim 56 where the sample is blood.

59. A method of detecting the presence of an analyte in a sample comprising binding an electrically readable particle to the analyte to produce a change in an electrical property of an electrical circuit and detecting the change in said electrical property using a pair of moveable electrodes wherein, in the presence of the analyte, the particles bind to at least one electrode.

60. The method of Claim 59 where the electrical circuit includes an electrode made of metal.

61. The method of Claim 60 where the metal is gold, silver, or platinum.

62. The method of Claim 59 where the electrical circuit includes an electrode made of a semiconductor.

63. The method of Claim 62 in which the semiconductor is doped silicon.

64. The method of Claim 59 where the electrical circuit includes an electrode made of a photoconductor.

65. The method of Claim 64 where the photoconductor is zinc sulfide, zinc selenide, cadmium sulfide, or cadmium selenide.
66. The method of Claim 59 where the electrically readable particles comprise a metal.
67. The method of Claim 66 where the metal is gold, silver, or platinum.
68. The method of Claim 59 where the electrically readable particles are made of graphite.
69. The method of Claim 59 where the electrically readable particles are made semiconductive material.
70. The method of Claim 69 where the semiconductive material is doped silicon.
71. The method of Claim 59 where the electrically readable particles are made of a photoconductor.
72. The method of Claim 71 where the photoconductor is zinc sulfide, zinc selenide, cadmium sulfide, or cadmium selenide.
73. The method of Claim 59 where the electrode is movable.
74. The method of claim 73 in which the said electrode is brought into physical contact with the electrically readable particles.

75. A method of claim 74 in which non-conductive particles are used as spacers between a pair of electrodes.

76. The method of Claim 59 where the electrical circuit includes a pair of electrodes, with the particles being bound to one electrode, and a physical element preventing the direct contact between the electrodes.

77. The method of Claim 76 where the physical element comprises ridges.

78. The method of Claim 76 where the physical element comprises hillocks.

79. The method of Claim 59 where the particles have a diameter of from 1 nanometer to 1 millimeter

80. The method of Claim 59 where the area of each electrodes exceeds 0.5 square micrometer.

81. The method of Claim 80 where the area of the electrodes have a range of from 10 square micrometers to 10 square millimeters.

82. An instrument for detecting in a sample the presence of a specific electrolyte, including

a port through which the sample is fed into the instrument, first and second electrodes, said first electrode including a surface portion that collects thereat said specific electrolyte, said first electrode being at a first polarity, and said second electrode including a surface portion that collects thereat electrolytes other than said

specific electrolyte, said second electrode being at a second polarity opposite said first polarity,

said electrodes having

a first position where they are separated a sufficient distance apart to enable the sample to move there between and to suppress an ion current there between and

a second position where the electrodes are sufficiently close to each other to establish an ion current,

a detection circuit, including the electrodes, that has a first state when the specific electrolyte is absent from the sample and a second state when the specific electrolyte is present in the sample, and

a signaling device that provides an indication when the detection circuit is in the second state.

83. An instrument for detecting in a sample the presence of a specific small molecule, including

a port through which the sample is fed into the instrument,

first and second electrodes, said first electrode including a surface portion that collects thereat said specific small molecule, said first electrode being at a first polarity, and said second electrode including a surface portion that collects thereat small molecules other than said specific small molecule, said second electrode being at a second polarity opposite said first polarity,

said electrodes having

a first position where they are separated a sufficient distance apart to enable the sample to move there between and to suppress an ion current there between, and

a second position where the electrodes are sufficiently close to each other to establish an ion current,

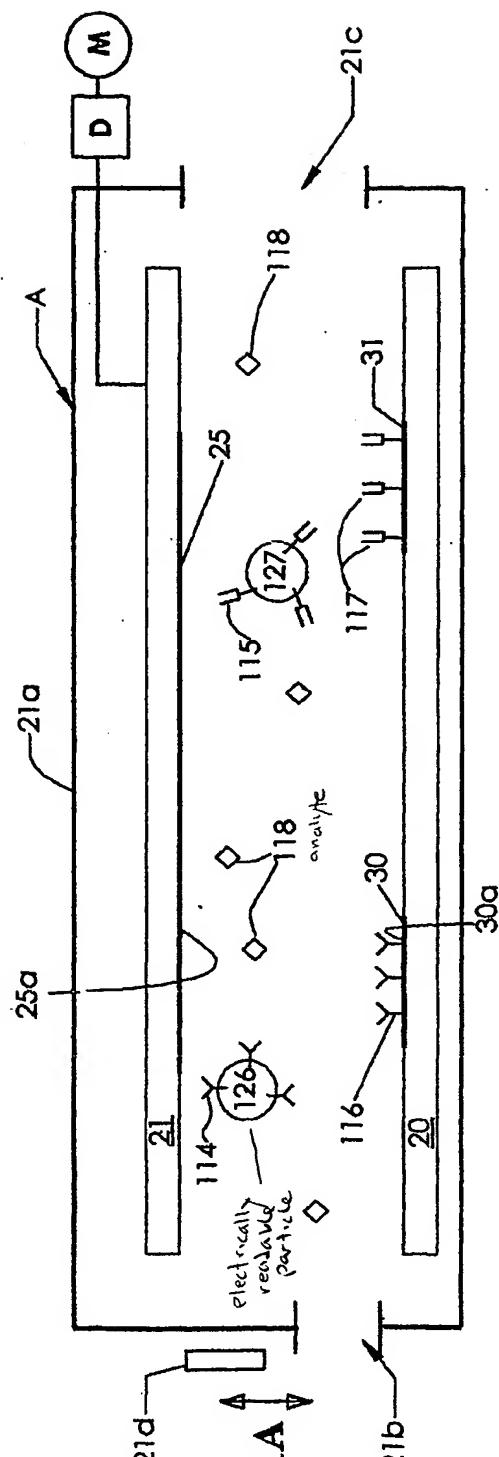
a detection circuit, including the electrodes, that has a first state when the specific small molecule is absent from the sample and a second state when the specific small molecule is present in the sample, and

a signaling device that provides an indication when the detection circuit is in the second state.

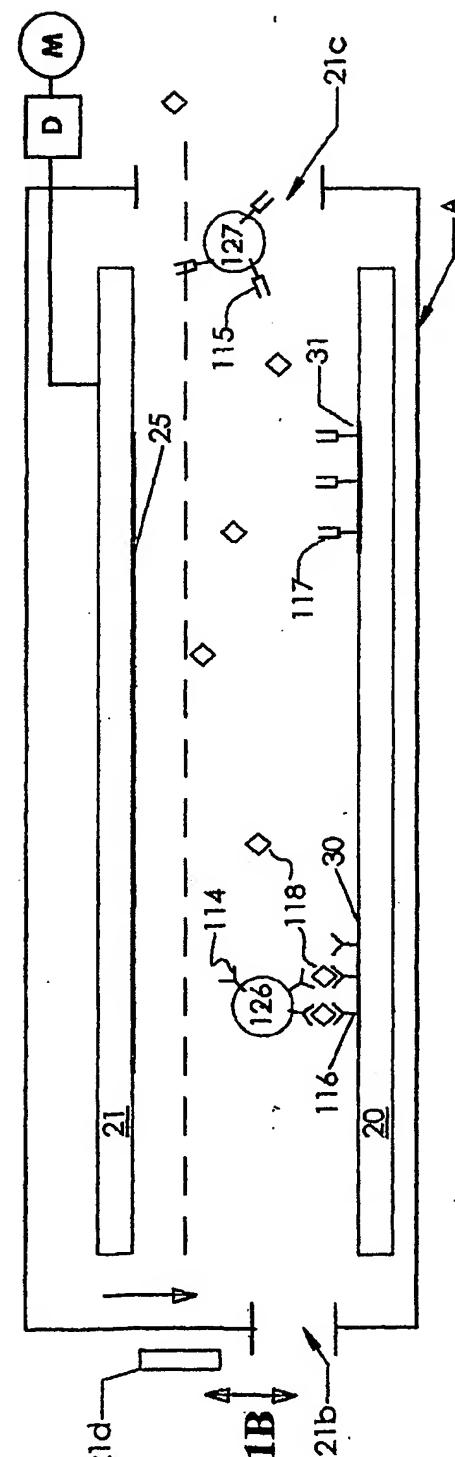
84. A method of detecting the presence of an analyte in a whole blood sample including particles having a size of 500 nanometers or more, comprising the steps of

(a) binding an electrically readable particle to the analyte to produce a change in an electrical property of an electrical circuit and detecting the change in said electrical property using a pair of electrodes spaced apart a distance of no greater than 1000 nanometers wherein, in the presence of the analyte, the particles bind to at least one electrode, and

(b) prior to step (a) filtering from the sample the particles.

**Fig. 1A**

1/30

**Fig. 1B**

?/6 pressure is
applied to 126

SUBSTITUTE SHEET (RULE 26)

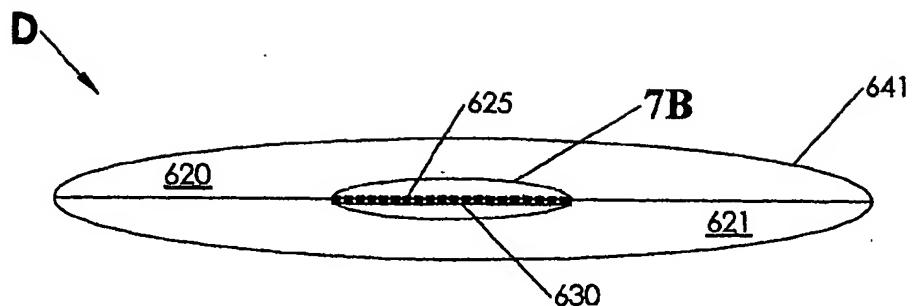


Fig. 7A

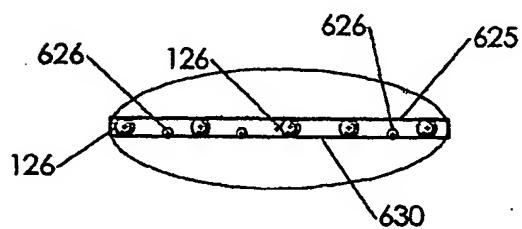


Fig. 7B

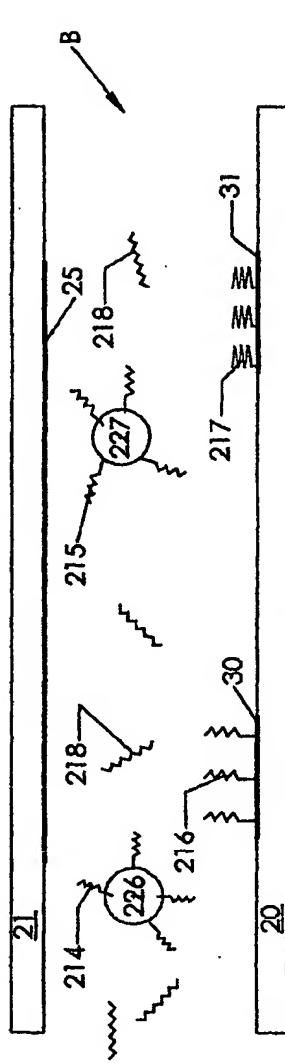


Fig. 2A

3/30

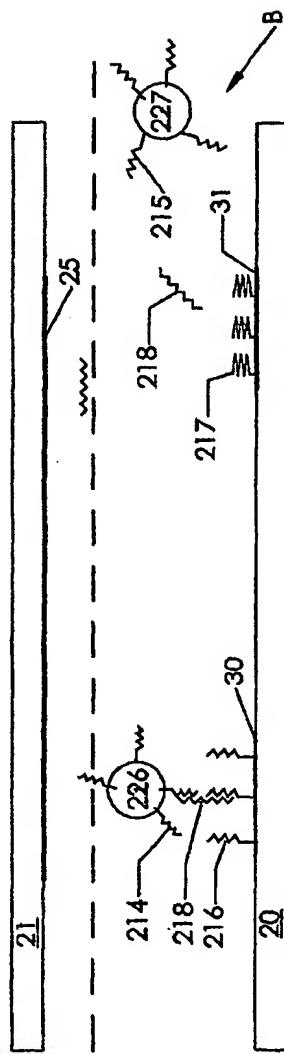
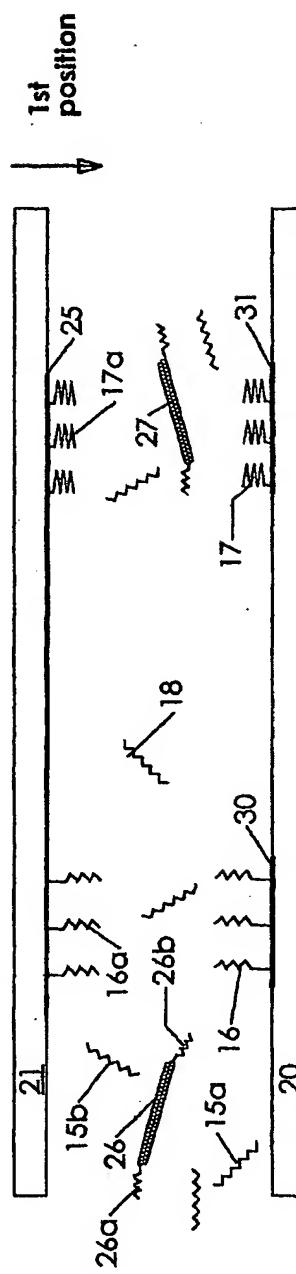
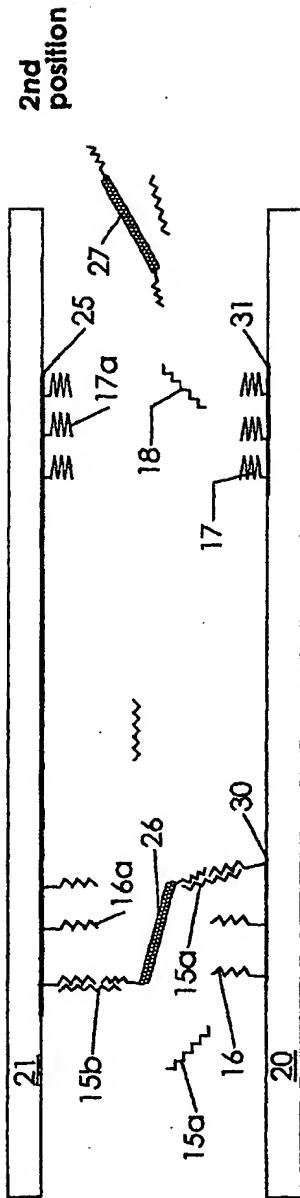
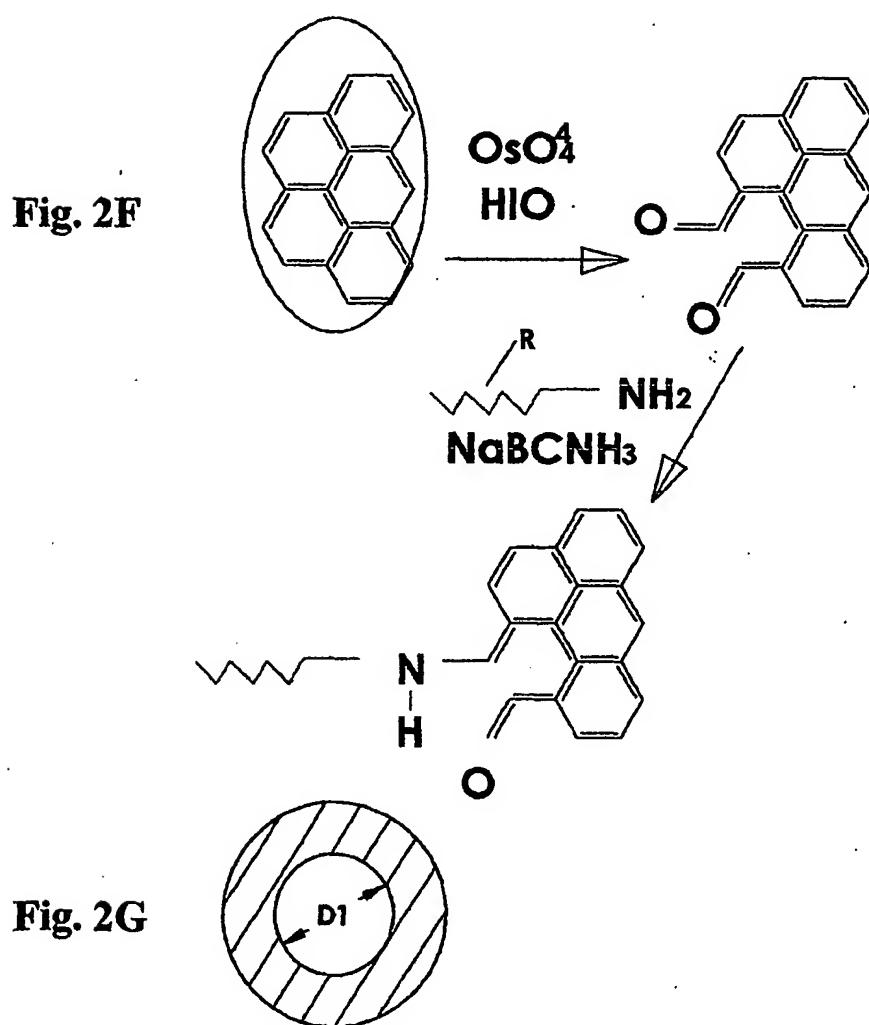
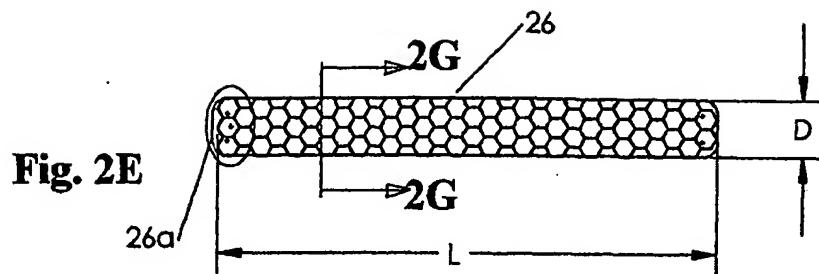


Fig. 2B

SUBSTITUTE SHEET (RULE 26)

**Fig.2C****Fig.2D**



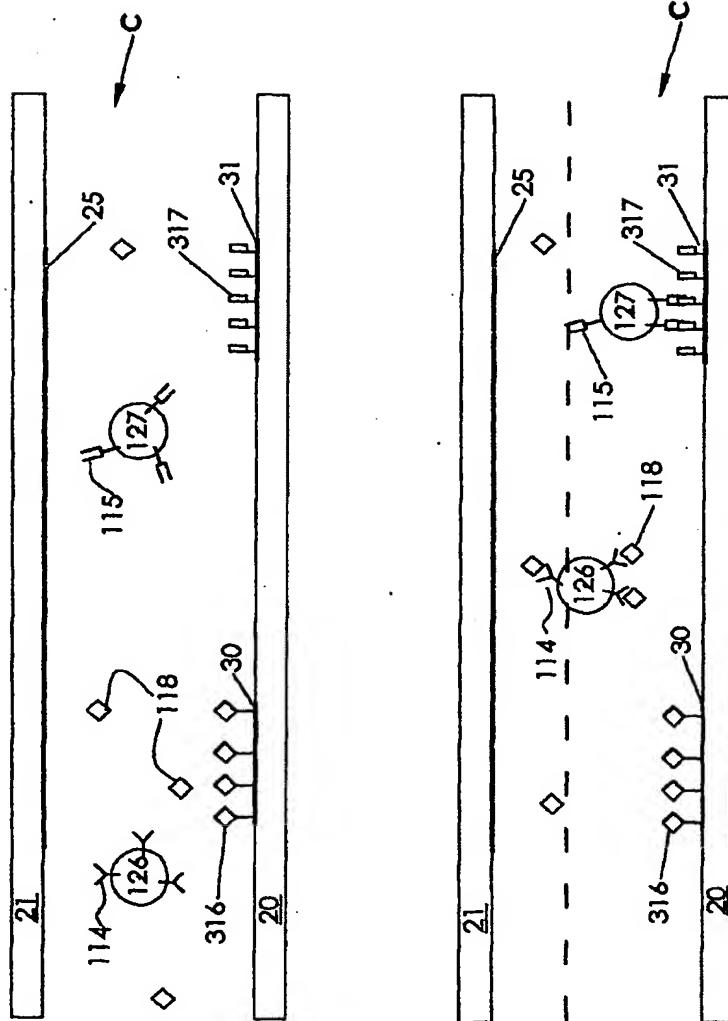


Fig. 3A

Fig. 3B

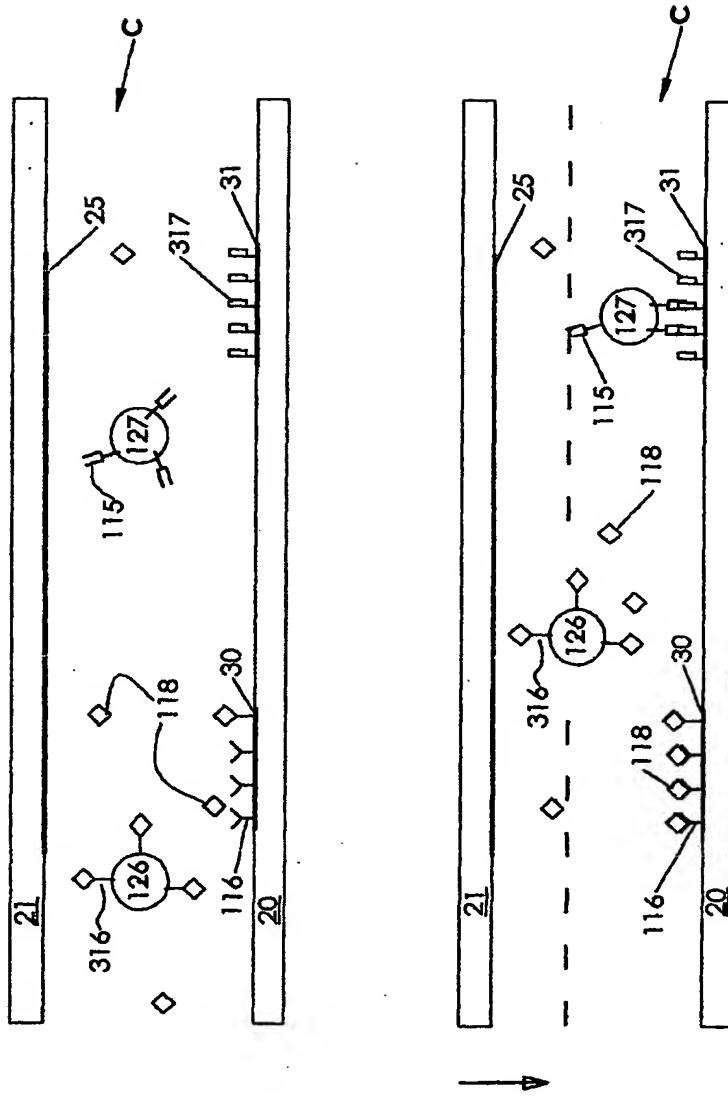


Fig. 3C

Fig. 3D

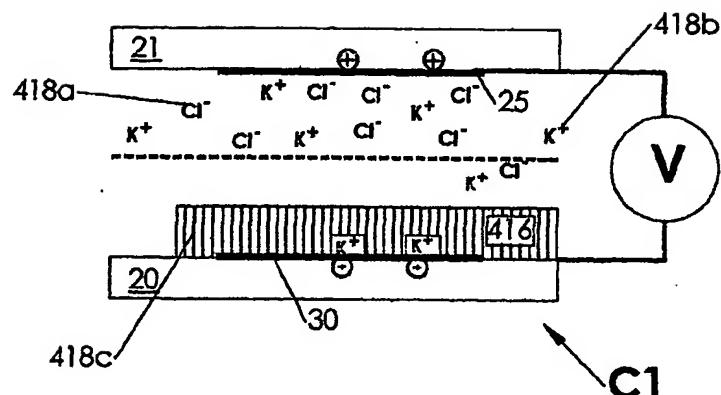


Fig. 4

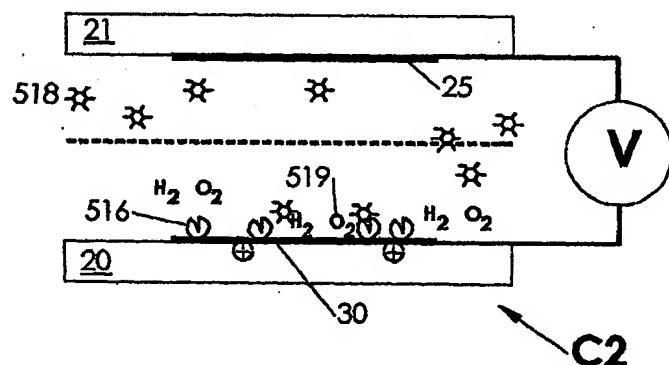
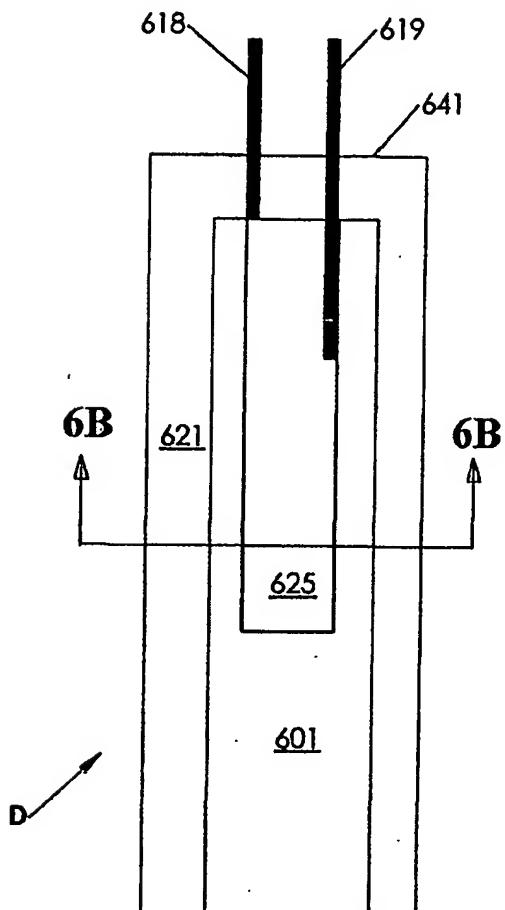
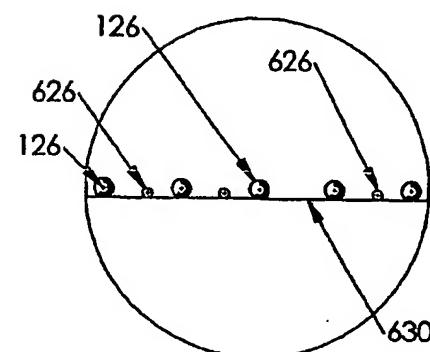
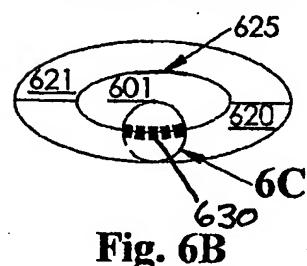


Fig. 5

SEARCHED

Top View**Fig. 6A****Cross Section****Fig. 6C**

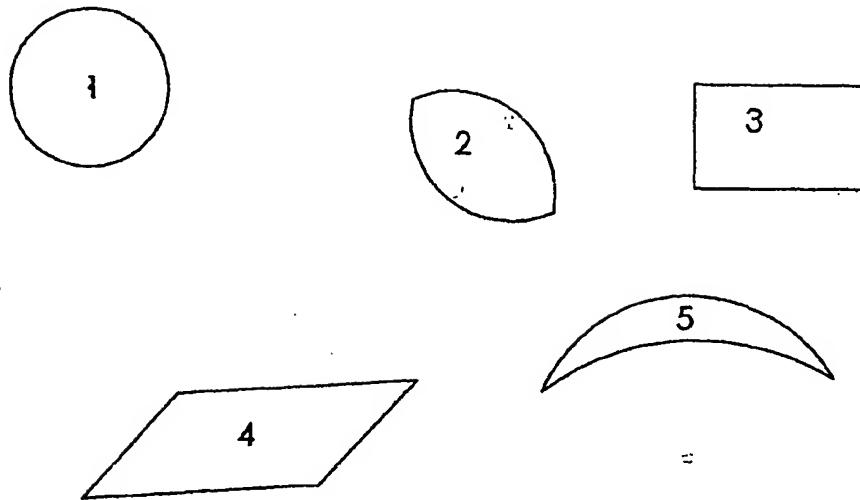


Fig. 8A

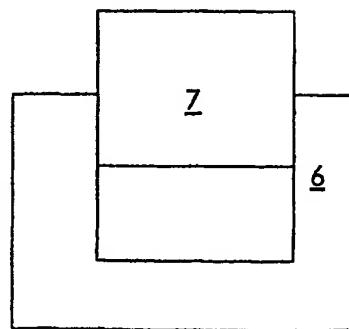


Fig. 8B

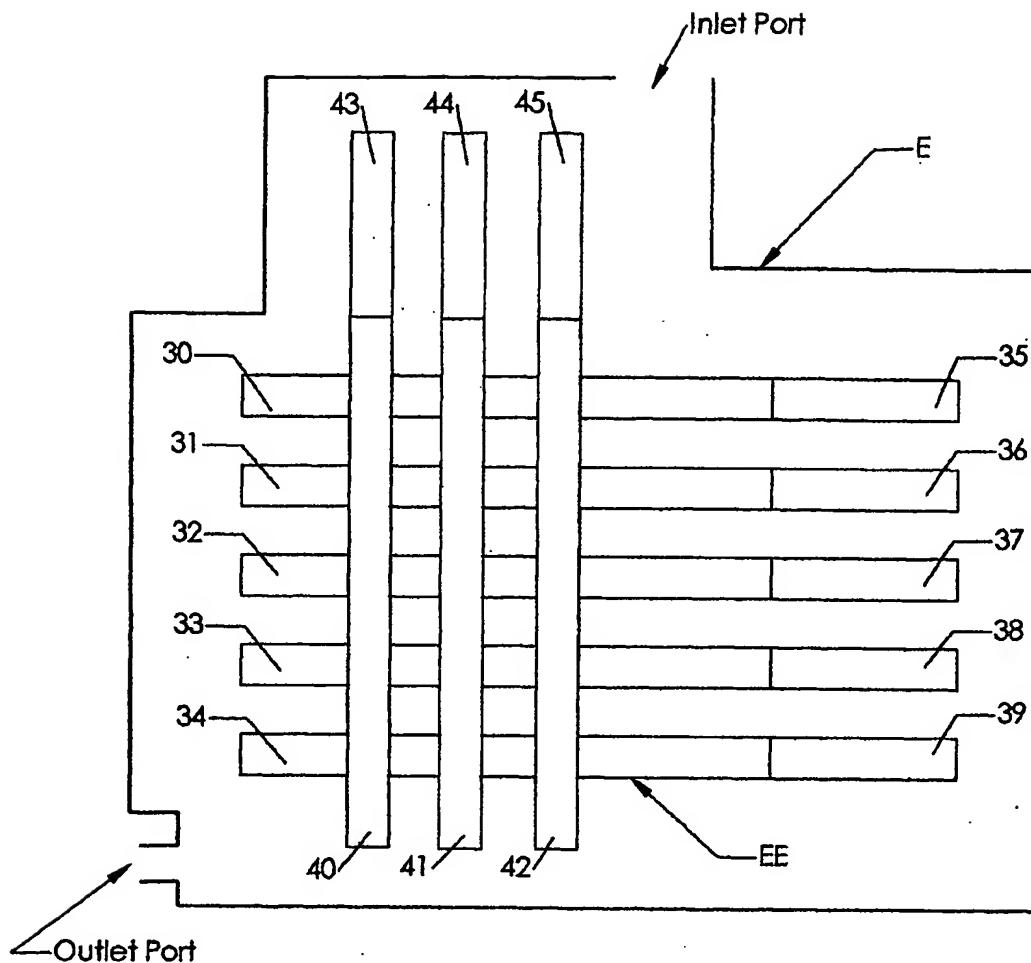


Fig. 9

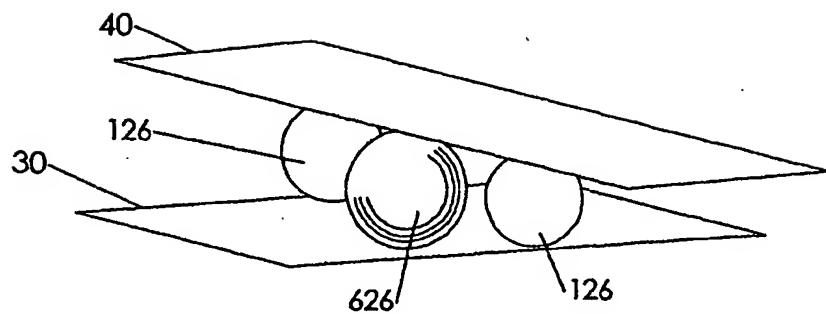


Fig. 10

12/30

SUBSTITUTE SHEET (RULE 26)

9/26/2006, EAST Version: 2.1.0.14

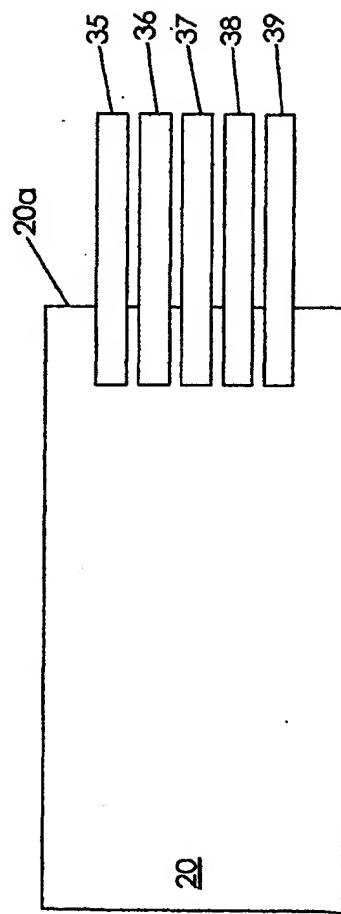


Fig. 11A

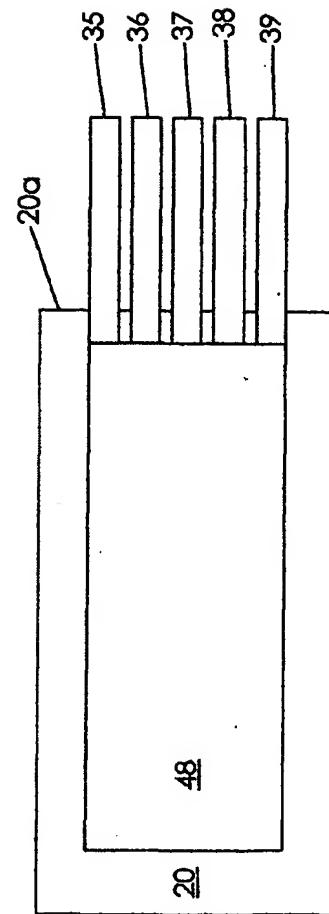


Fig. 11B

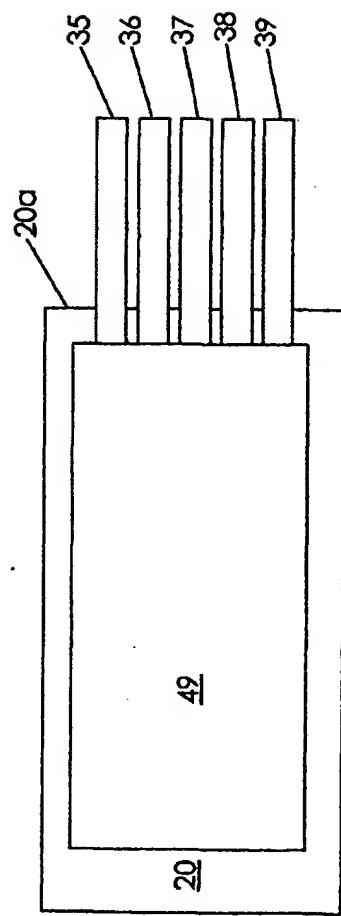


Fig. 11C

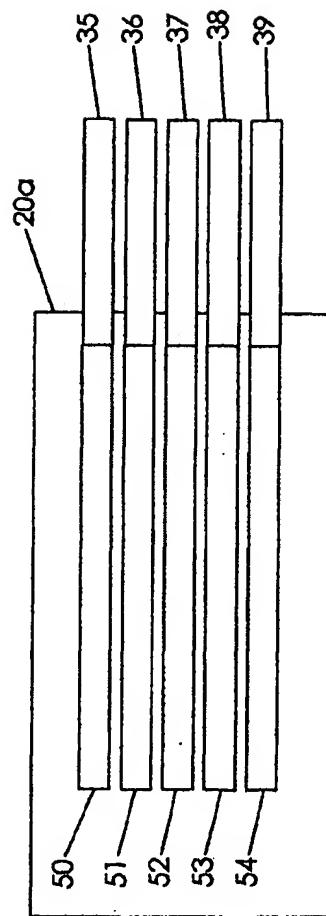


Fig. 11D

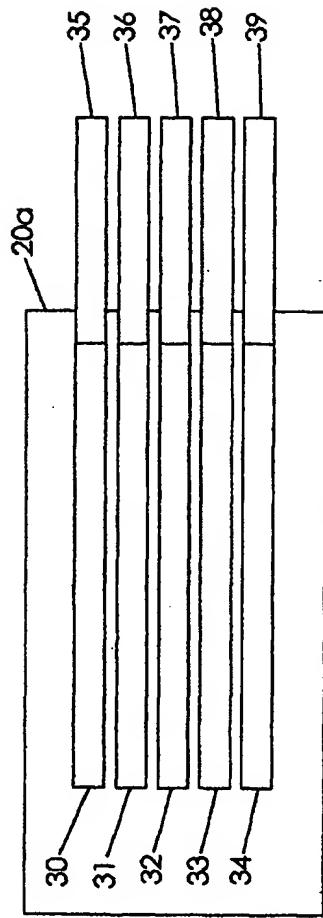


Fig. 11E

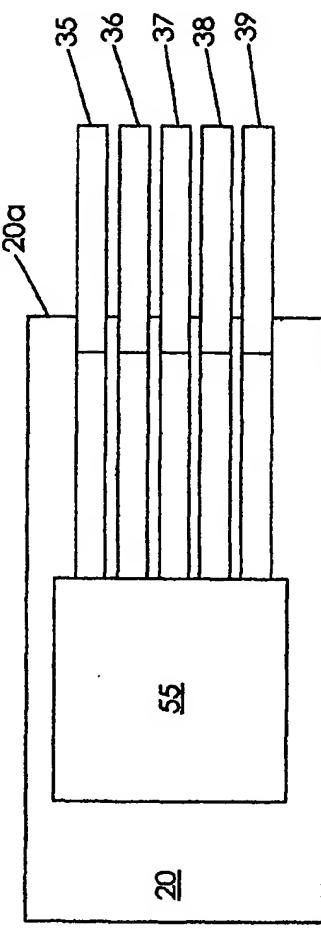


Fig. 11F

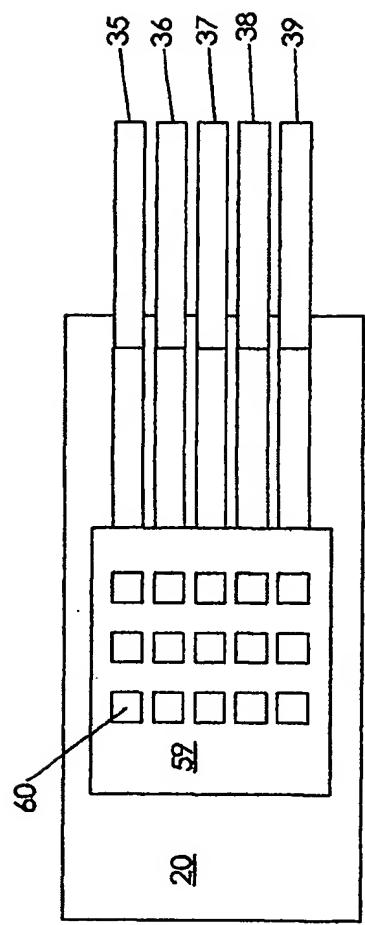


Fig. 11G

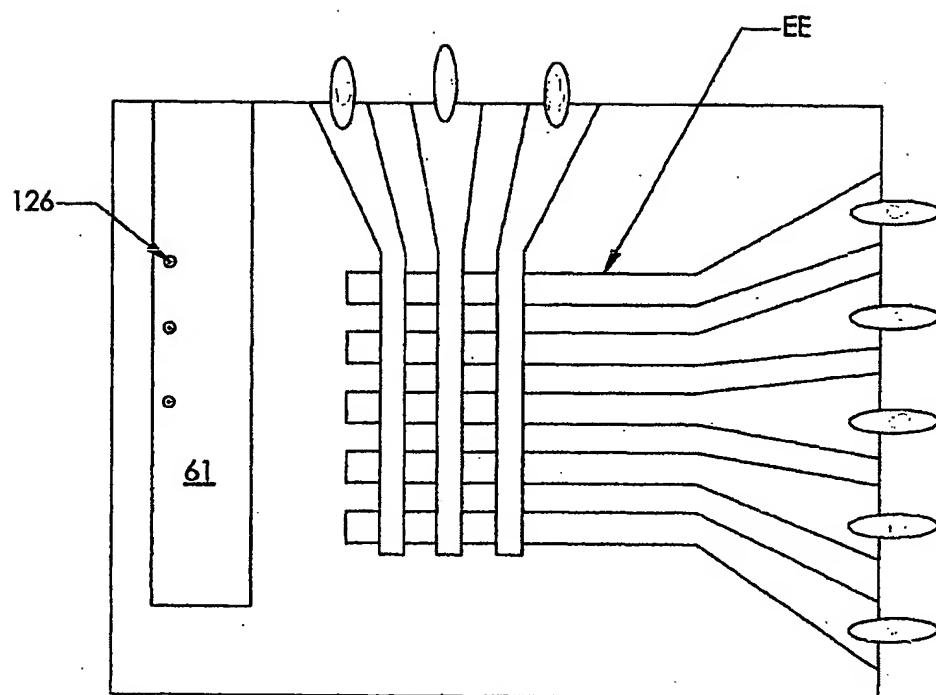


Fig. 12

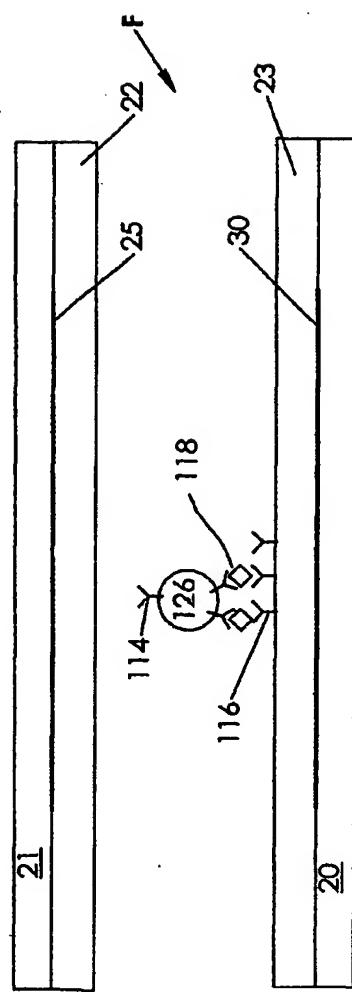


Fig. 13A

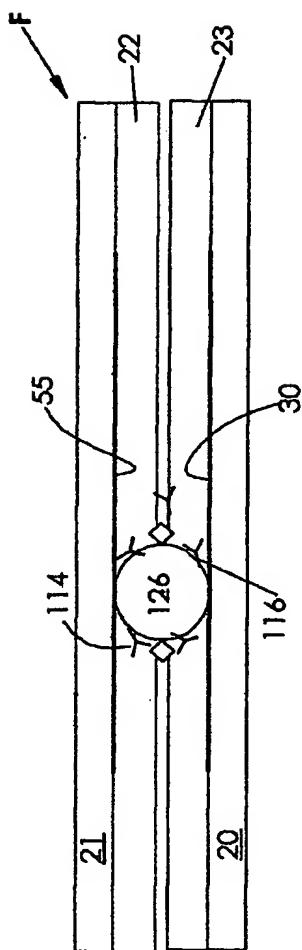
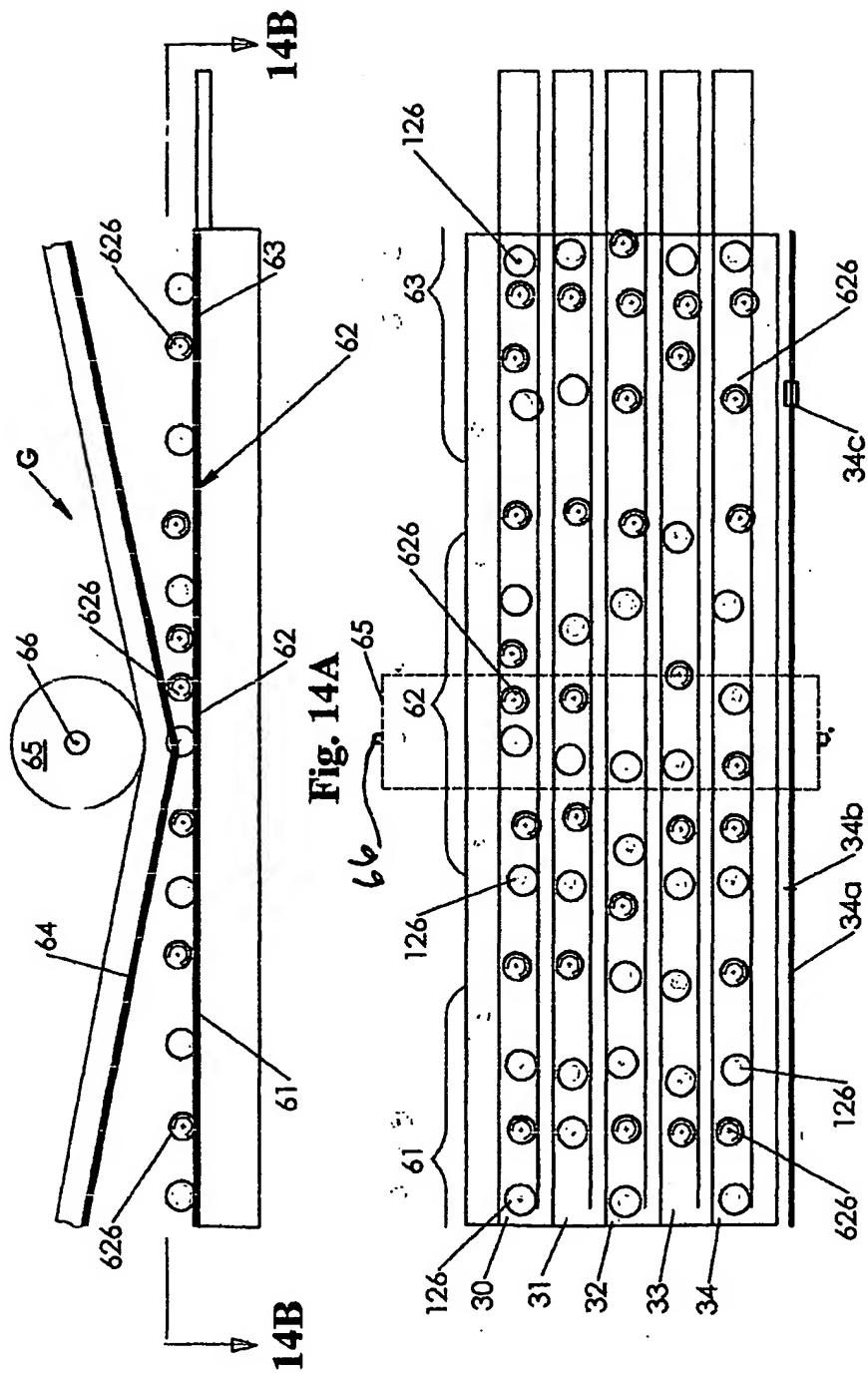
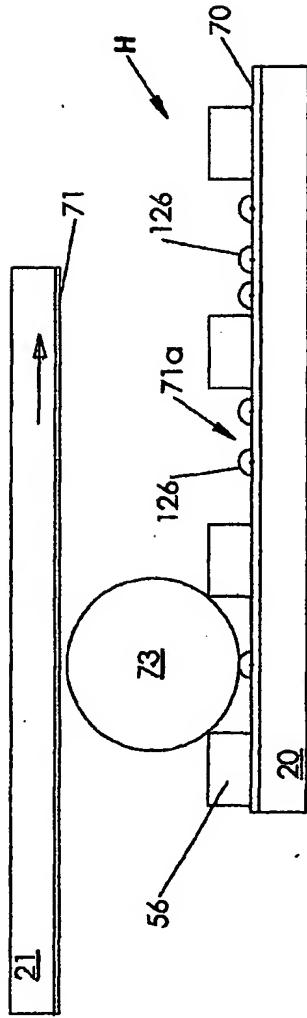
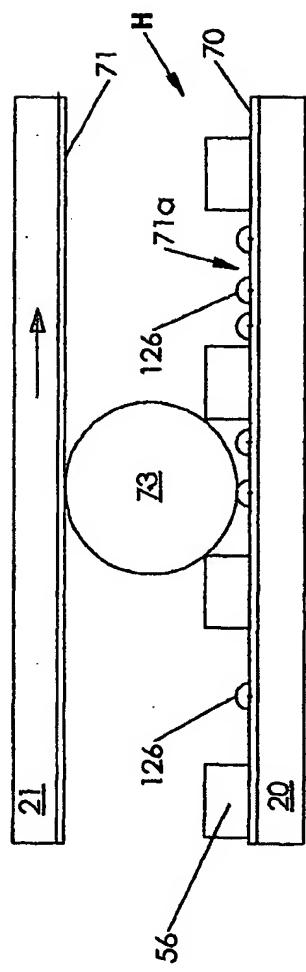
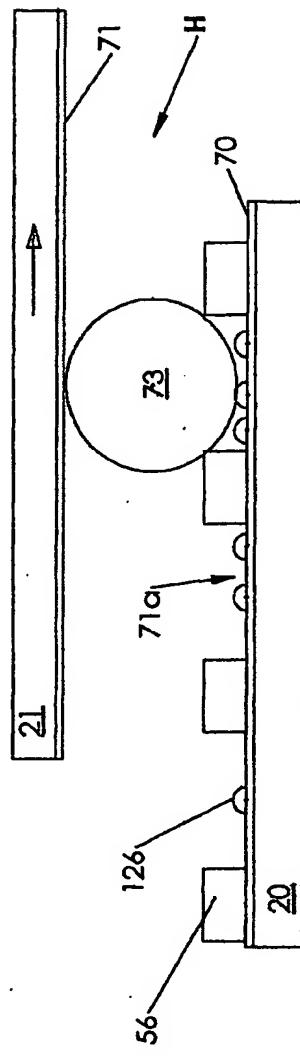
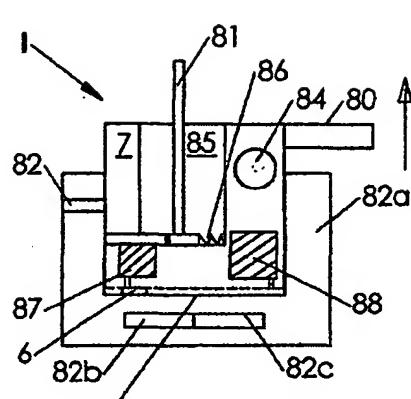
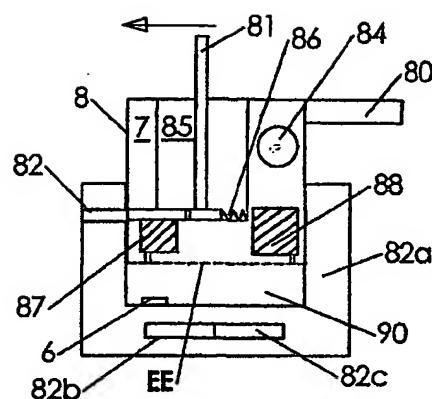
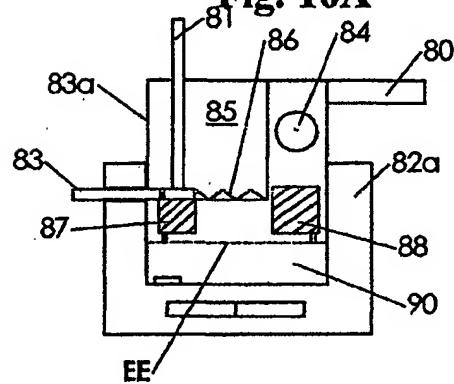
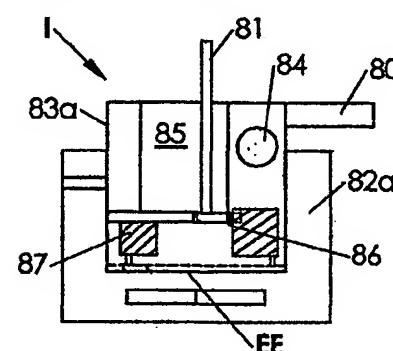
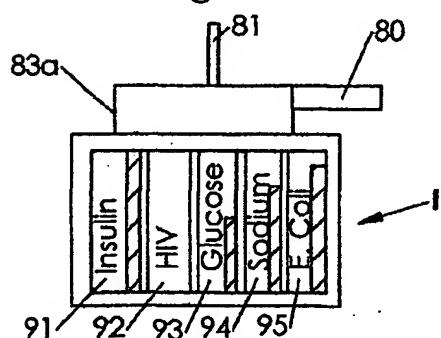
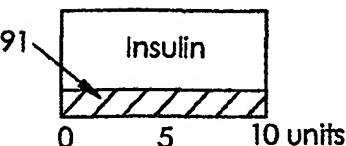


Fig. 13B



**Fig. 15A****Fig. 15B****Fig. 15C**

**Fig. 16A****Fig. 16B****Fig. 16C****Fig. 16D****Fig. 16E****Fig. 16F**

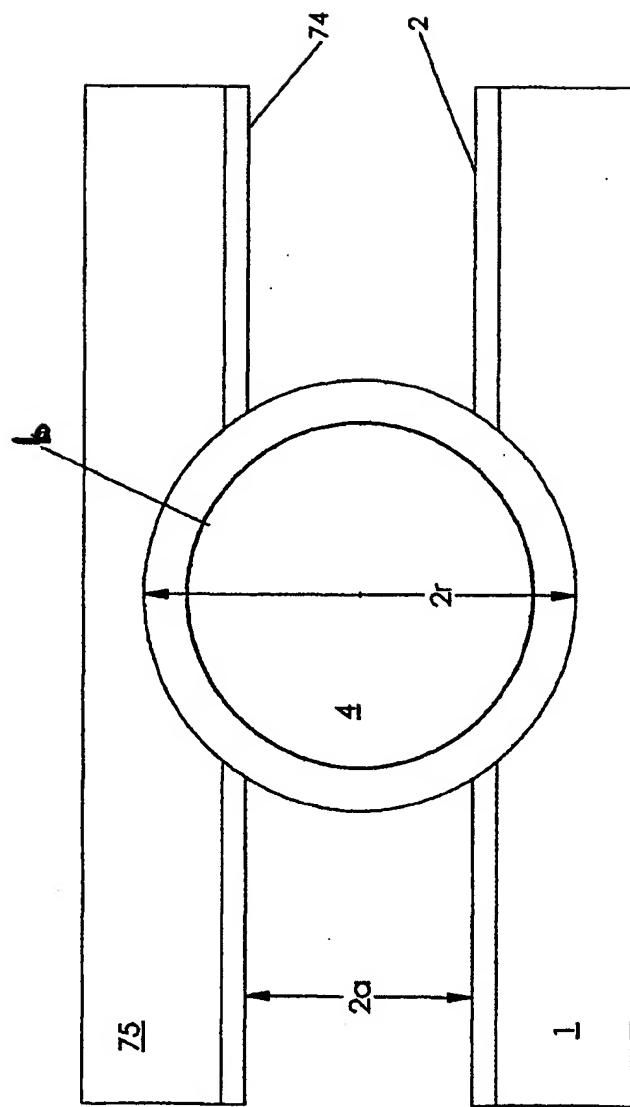
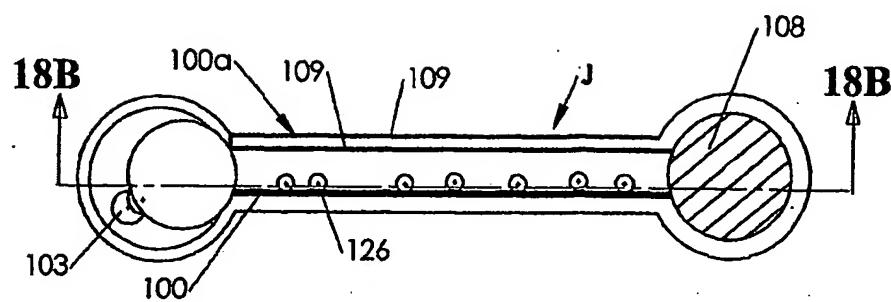
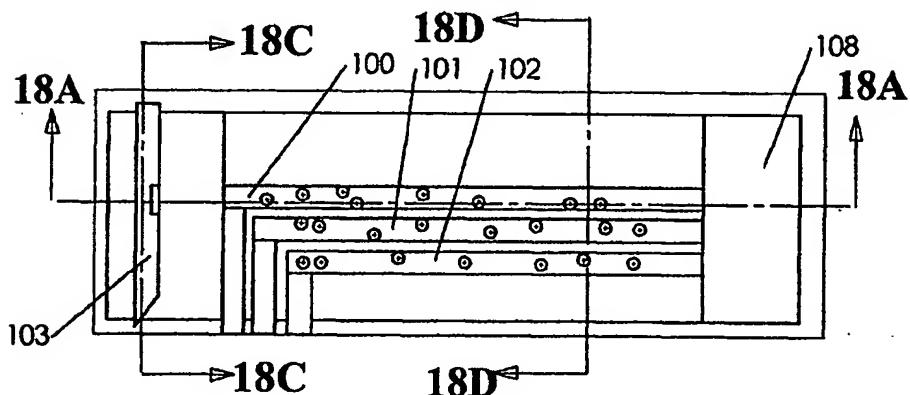
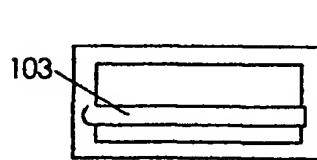
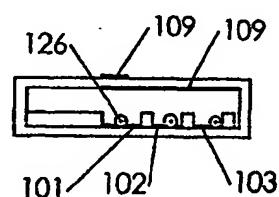


Fig. 17

**Fig. 18A****Fig. 18B****Fig. 18C****Fig. 18D**

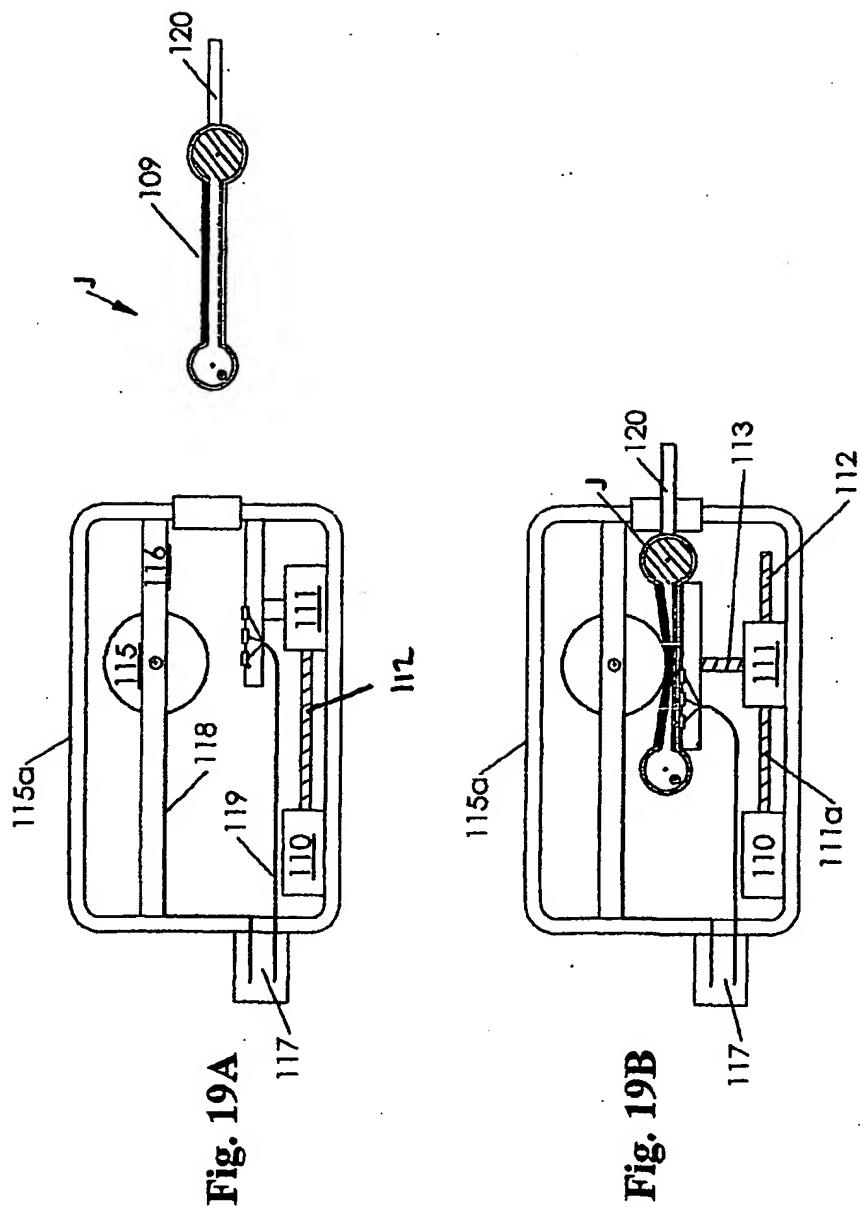


Fig. 19A

Fig. 19B

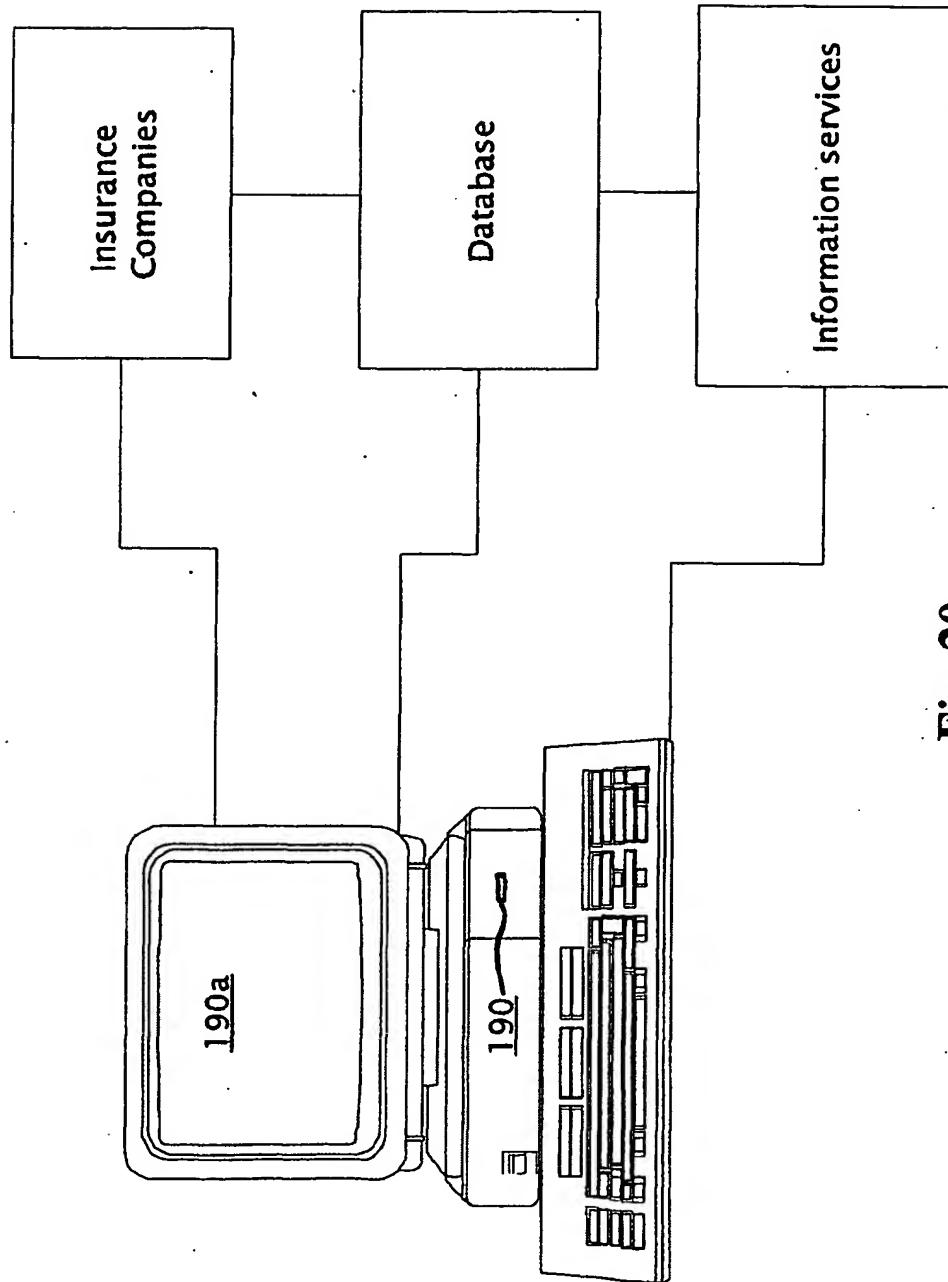
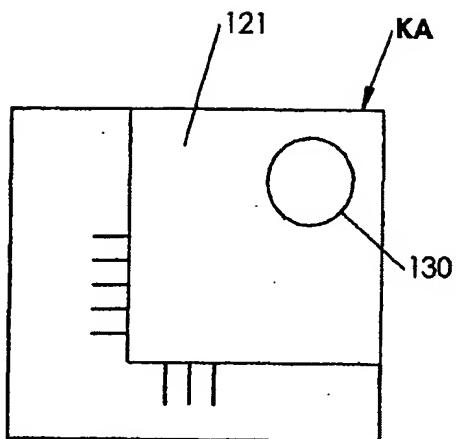
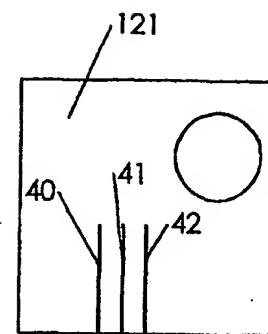
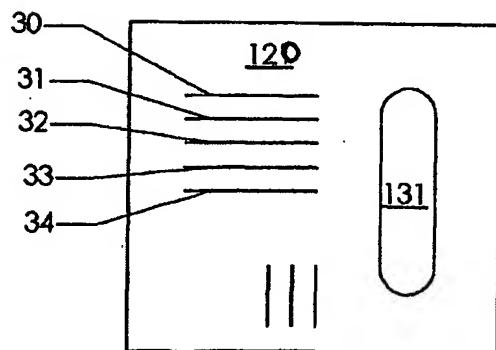


Fig. 20

**Fig. 21A****Fig. 21B****Fig. 21C**

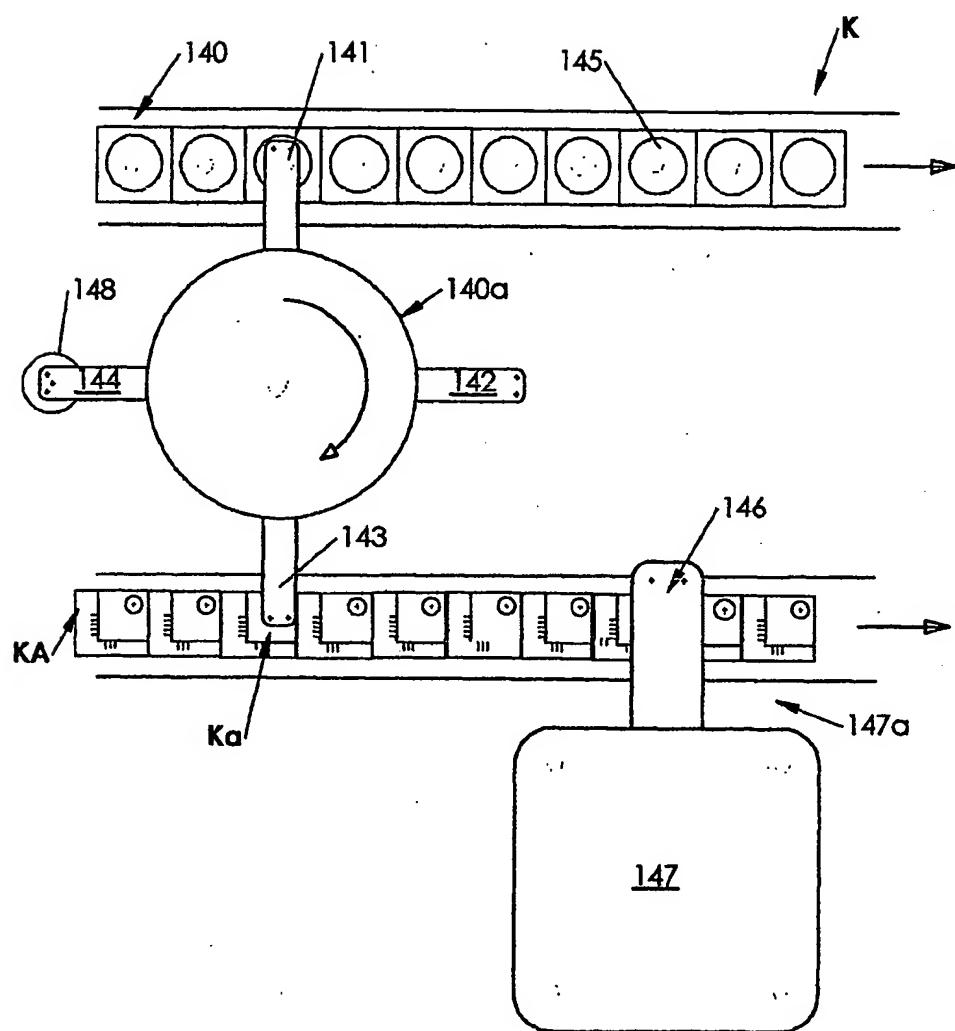


Fig. 22

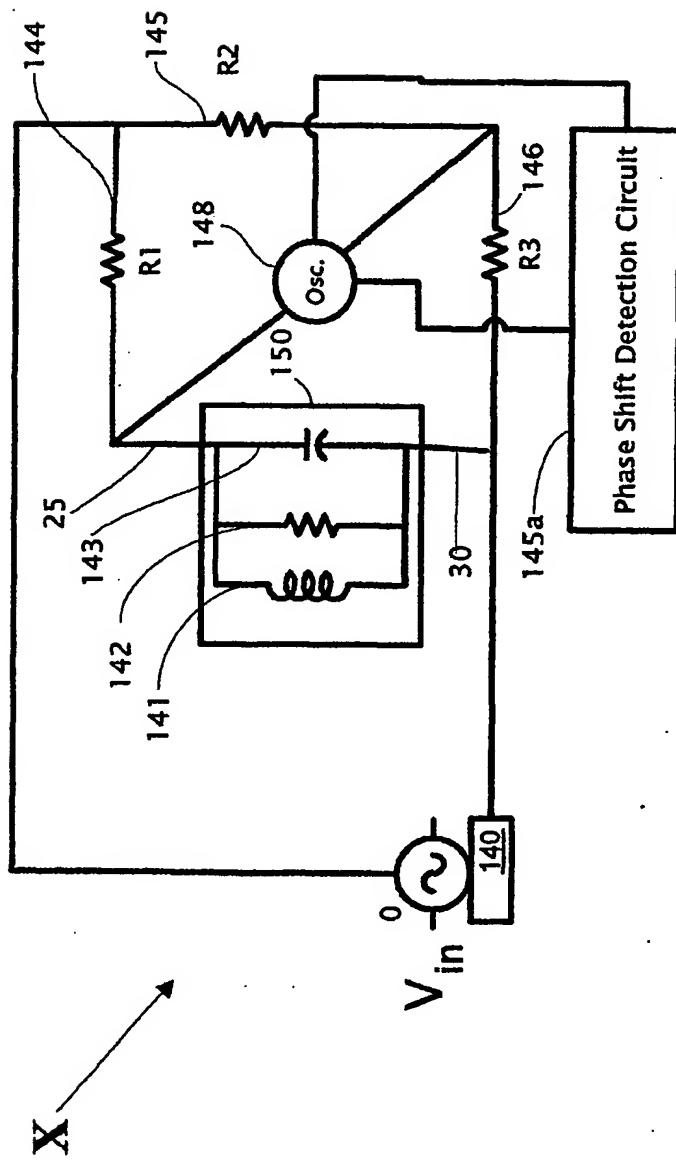


Fig. 23

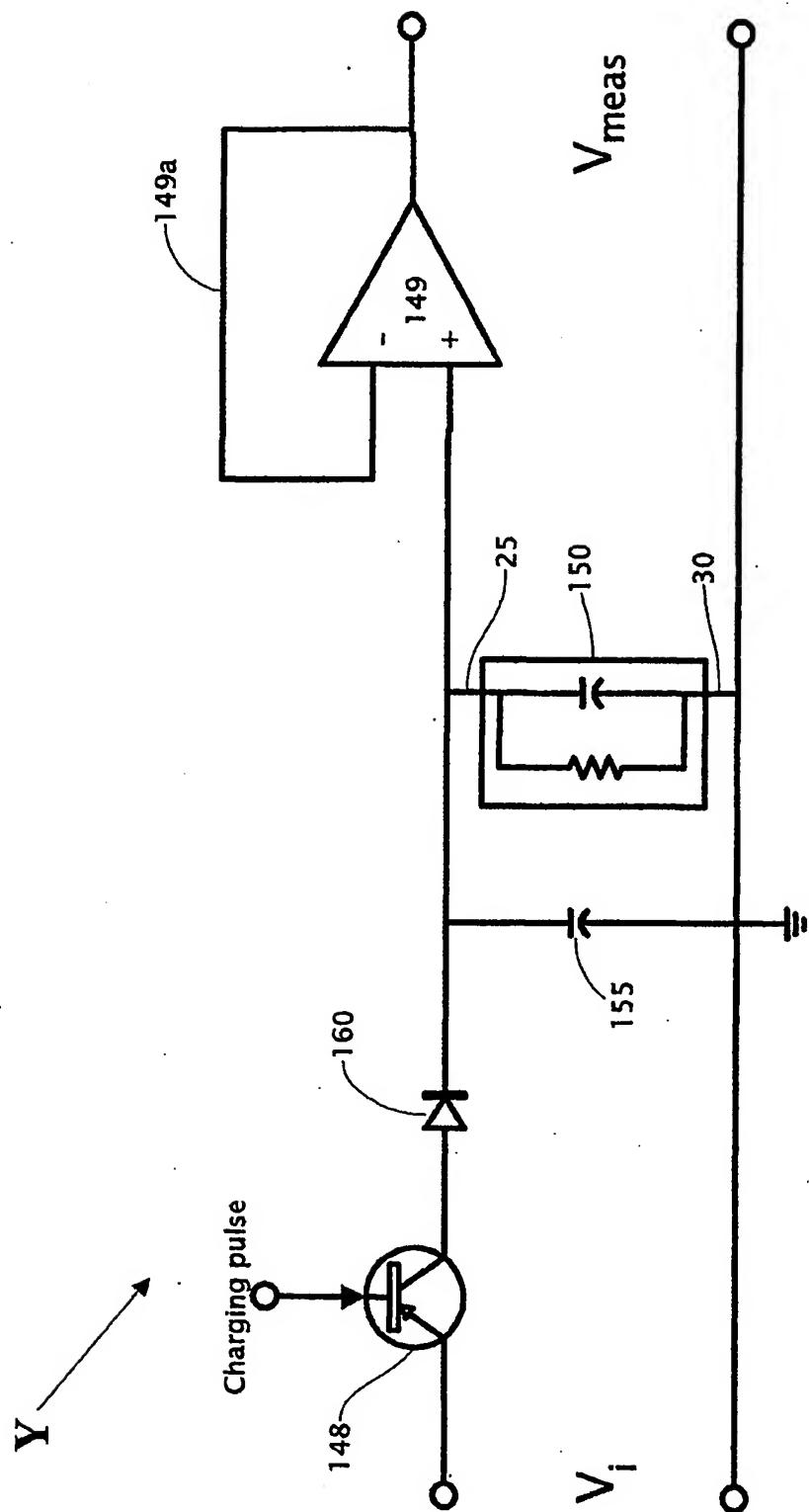


Fig. 24

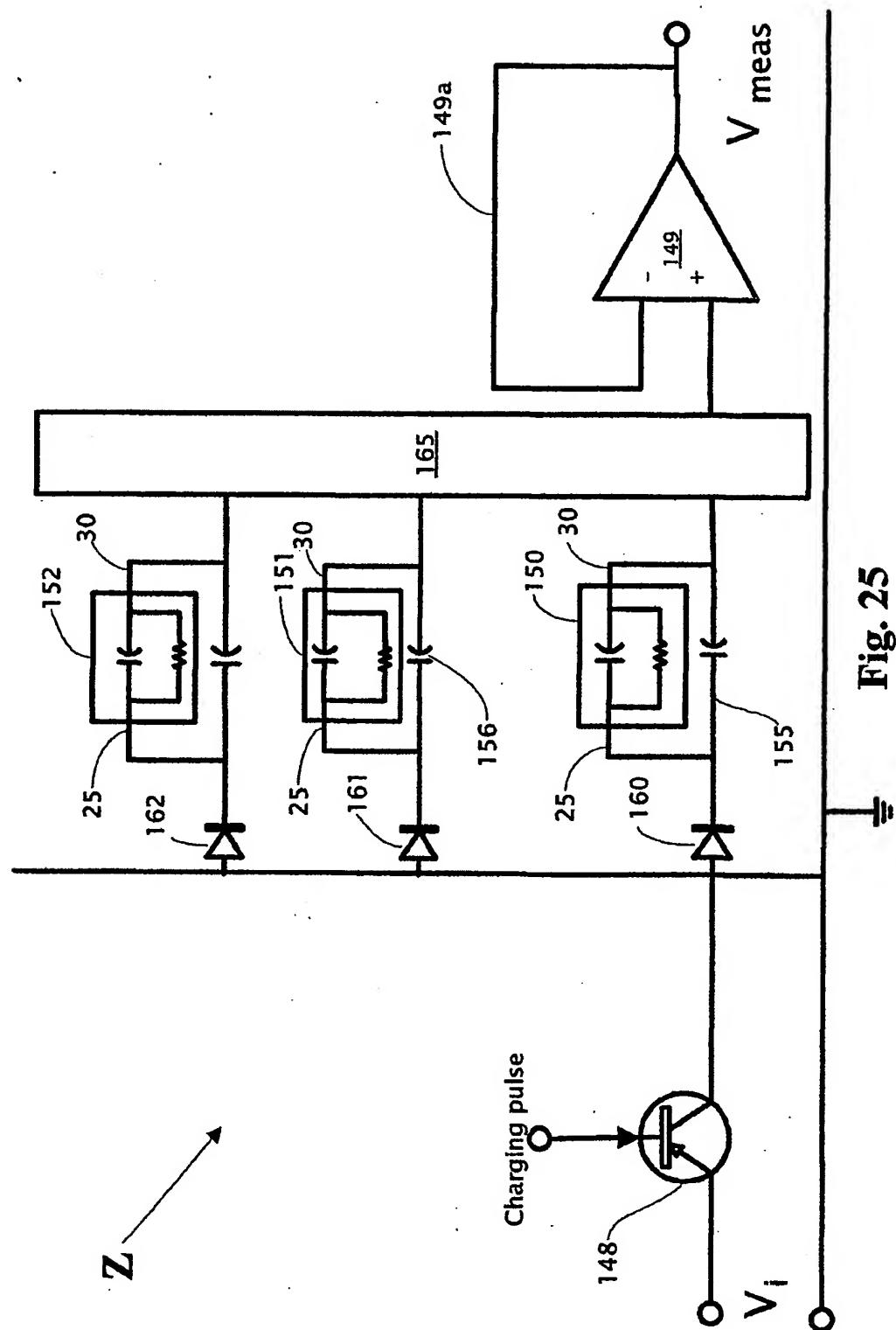


Fig. 25

30/30
SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/00461

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :G01N 27/12

US CL :~~422/82.01, 82.02; 436/525, 526, 537, 540, 151~~

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : ~~422/82.01, 82.02; 436/525, 526, 537, 540, 151~~

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|--|-----------------------|
| A | US 6,100,045 A (VEN ES) 08 August 2000, entire document. | 1-84 |
| A | US 5,728,590 A (POWELL) 17 March 1998, entire document. | 1-84 |
| A | US 5,491,097 A (RIBI et al) 13 February 1996, entire document. | 1-84 |
| A | US 5,284,748 A (MROCKOWSKI et al) 08 February 1994, entire document. | 1-84 |
| A | US 5,137,827 A (MROCKOWSKI et al) 11 August 1992, entire document. | 1-84 |

Further documents are listed in the continuation of Box C. See patent family annex.

| | | |
|--|--|--|
| * Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | |
| "E" | earlier document published on or after the international filing date | "X" |
| "L" | document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "O" | document referring to an oral disclosure, use, exhibition or other means | "Y" |
| "P" | document published prior to the international filing date but later than the priority date claimed | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| | | "Z" |
| | | document member of the same patent family |

| | |
|---|--|
| Date of the actual completion of the international search | Date of mailing of the international search report |
| 29 APRIL 2002 | 14 MAY 2002 |
| Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 505-9930 | Authorized officer JEFFREY R. SNAY Telephone No. (703) 508-0681 Jean Provost Patent Agent OVI |

Form PCT/ISA/310 (second sheet) (July 1998)*